



Patent Application No. 09/920,017
Atty. Dkt. No. 054707-0355

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Applicant: Gregory S. HAMILTON et al.

Title: CARBOXYLIC ACIDS AND
CARBOXYLIC ACID
ISOSTERES OF N-
HETEROCYCLIC COMPOUNDS

Appl. No.: 09/920,017

Filing Date: 8/2/2001

Examiner: Celia C. Chang

Art Unit: 1625

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BRIEF ON APPEAL

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Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

Sir:

This Brief on Appeal follows the Notice of Appeal filed September 2, 2003. It is timely, as it is filed Tuesday, December 2, 2003, and accompanied by a Petition for an one-month Extension of Time and the fee of \$55.00 under 37 C.F.R. § 1.17(a)(2).

Under the provisions of 37 C.F.R. § 1.192, this Appeal Brief is being filed in triplicate together with a check in the amount of \$165.00 covering the Rule 17(c) - appeal fee. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

REAL PARTY IN INTEREST

The real party in interest is GPI NIL Holdings, Inc. (All rights in U.S. Application No. 09/920,017 are assigned to GPI NIL Holdings, Inc. The assignment for recordation will follow.)

RELATED APPEALS AND INTERFERENCES

Appellant, Appellant's legal representative, and Assignee know of no other appeals or interferences that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

STATUS OF CLAIMS

Claims 1-82 are all the claims in the application. Claims 1-3, 5-7, 9-11, and 13 are pending. Claims 4, 8, 12, and 14-82 are cancelled. Claims 1-3, 5-7, 9-11, and 13 are appealed.

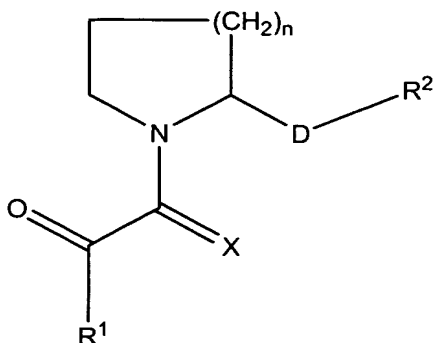
STATUS OF AMENDMENTS

All amendments were entered.

To incorporate a nomenclature suggestion offered by the examiner, the compound recited in claim 6 should read 1-{2-[5-(4-fluorophenyl)(1,2,4-oxadiazol-3-yl)] pyrrolidin-1-yl}-3,3-di-methylpentane-1,2-dione instead of 1-{2-[3-(4-Fluorophenyl)(1,2,4-oxadiazol-5-yl)] pyrrolidinyl}-3,3-di-methylpentane-1,2-dione.

SUMMARY OF INVENTION

The invention includes compounds, e.g., of Formula (I)



and pharmaceutical compositions. Specification at page 20, lines 10 - *et seq.*

ISSUES

- A. Whether claims 1, 2, 5, 6, 10, and 13 are unpatentable under 35 U.S.C. § 112, second paragraph.
- B. Whether claims 1-2 and 9 are unpatentable under 35 U.S.C. § 103(a) over Bycroft *et al.*, Burbaum *et al.*, Gold *et al.* and Hamilton.
- C. Whether claims 1-3, 5, 9-11 and 13 are unpatentable under 35 U.S.C. § 102(f) over WO 99/14998.
- D. Whether claims 1, 2, 6, 7, 9, and 10 are unpatentable under 35 U.S.C. § 102(e), (f) or (g) as anticipated by Brumby *et al.* or Kato *et al.* supplemented by Andersen *et al.*
- E. Whether claims 1-3 and 9-11 are unpatentable under 35 U.S.C. § 103(a) over U.S. Patent Nos. 5,859,031, 5,945,441, 6,177,455 and 6,291,510 in view of Silverman or Bungaard, further in view of U.S. Patent Nos. 5,801,187 and 6,218,544.

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- F. Whether claims 1-3 and 9-11 are unpatentable under the judicially created doctrine obviousness-type double patenting over U.S. Patent Nos. 5,859,031, and 6,291,510 in view of Silverman or Bungaard further in view of U.S. Patent Nos. 5,801,187 and 6,218,544.
- G. Whether the refusal to examine the entire scope of the claims is an improper rejection and should be reversed, according to *In re Haas*, 198 USPQ 334 (CCPA 1978).

GROUPING OF CLAIMS

For the purpose of this appeal only, the claims stand or fall together for each ground of rejection which Appellant contests and which applies to a group of two or more claims.

ARGUMENT

The United States Patent and Trademark Office (U.S.P.T.O.) has the initial burden to present its prima facie case of unpatentability. *In re Oetiker*, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). The core factual underpinnings of case of unpatentability cannot be conclusions based on anything but concrete evidence in the record. *In re Zurko*, 59 USPQ2d 1693 (Fed. Cir. 2001). Indeed, a factual finding that is material to patentability can neither stand if it is supported only by conclusory statements nor be resolved on the subjective belief of an examiner. *In re Lee*, 277 F.3d 1338, 1346, 61 USPQ2d 1430, 1433 (Fed. Cir. 2002).

Throughout the prosecution of the application on appeal the examiner has failed to supply evidence and explanation supporting a case of unpatentability. The examiner rejects all pending claims over 35 U.S.C. §§ 112, second paragraph, 102 and 103. However, in each instance, the examiner neither points to any evidence of record nor supplies any explanation in support a case of unpatentability. In many cases, the examiner relies on sweeping statements, such as “enormous prior art,” without citing any individual reference. The examiner also relies on references which, by her own admission, received only a “cursory review.” (M.P.E.P. § 609 C(2) (“The Examiner will consider the documents in the same manner as other documents in office search files are considered by the Examiner while

conducting a search of the prior art in a proper field of search.”)¹ As a result, the following arguments focus on why the evidence and explanation of record fail to establish a prima facie case of unpatentability.

A. The rejection of claims 1, 2, 5, 6, 10, and 13 under 35 U.S.C. §112, second paragraph should be reversed, because the evidence and explanation fail to establish the claims are indefinite.

The rejection of claims 2, 5, 6, 10, and 13 under 35 U.S.C. §112, second paragraph is improper, because the claims are not indefinite. The second paragraph of section 112 requires only that the claims reasonably apprise those skilled in the art of the scope of the claimed invention. *See e.g. Miles Lab, Inc. v. Shandon, Inc.*, 27 U.S.P.Q.2d 1123 (Fed. Cir. 1993), *cert denied*, 510 U.S. 1100 (1994), *see generally* M.P.E.P. § 2173.02. Furthermore, it is the examiner who has the initial burden of demonstrating that one of skill in the art would not appreciate the metes and bounds of the claimed subject matter. M.P.E.P. § 706.03.

In this case, nevertheless, according to the examiner, “Claim 1 is ambiguous. It has been clearly delineated in the previous office action ‘what’ is examined for which the claim has not been limited to.” Office Action dated May 30, 2003. The examiner continues, stating:

the proviso conditions can only exclude anticipation but not obvious variations thereof such as homologs, one methyl addition compounds, isosteric replacements, position isomers etc. for which a tremendous amount of prior art existed and

¹ As per the examiner’s own notation, the references submitted by Appellants on the form PTO-1449 received on a “cursory review as submitted.” *See* form PTO-1449 considered by examiner on December 5, 2002 (Appendix B). The M.P.E.P. does not authorize a cursory review of documents submitted in an Information Disclosure Statement and it is certainly contrary to U.S.P.T.O. procedure to base a rejection on such references.

exhausted listing will not be made here but applicants are urged to consult the 26 pages of 1449 with all its references and compounds looking for homologs, one methyl addition compounds, isosteric replacements or position isomers which are well recognized *prima facie* structural obvious compounds over the provisoed compounds.

Id. In the text quoted above, the examiner seems to be alleging that the claims are indefinite because the limitations of the negative proviso of claim 1 does not exclude “obvious variations” of the excluded species. This reasoning is confusing in a section 112, second paragraph context and such a basis of rejection would be improper.

The examiner fails to meet the burden of demonstrating that one of skill in the art would not appreciate the metes and bounds of the claimed subject matter. Merely alleging that the proviso limitations are obvious and directing Applicants to consult the “26 pages of 1449 with all its references and compounds looking for” compounds which would render obvious the negative limitations of the claim 1 proviso has nothing to do with whether or not the claims reasonably apprise those skilled in the art of the scope of the claimed invention. Because the negative limitations of claim 1 clearly meet the reasonably apprised standard, and because the examiner has provided no explanation and evidence to the contrary, the rejection is improper.

Moreover, even if, *arguendo*, the proviso of claim 1 were “ambiguous,” that finding would be insufficient to render the claim indefinite. Indeed, a claim is not indefinite merely because it poses an issue of claim construction. *Exxon Research and Engineering Co. v. United States*, 265 F.3d 1371, 1375, 60 USPQ2d 1272, 1276 (Fed. Cir. 2001). A claim is definite if it is amenable to construction, however confusing that task may be. *Exxon*, 265 F.3d at 1375, 60 USPQ2d at 1276. In other words, if the meaning of the claim is discernible,

the claim avoids a rejection on indefiniteness grounds. *Id.* Because the meaning of claim 1 is discernible, claim 1 avoids a rejection on indefiniteness grounds. Accordingly, the rejection of claim 1 should be reversed.

Additionally, the examiner's rationale for rejecting claims 2 and 10 under 35 U.S.C. § 112, second paragraph also fails to meet the burden set out by the Court. The December 12, 2002, Office Action states: "Claim 2 and 10 are self-conflicting because claim 2 has the scope that 'R₂ is carbocycle...containing CH₂...' which will not include phenyl yet on page 125, the R₂ moiety was either phenyl or phenyl tautomer." Office Action dated December 12, 2002, at page 3. Appellants maintain that the claim would reasonably apprise those skilled in the art of the scope of the claimed invention.

Claim 2 depends from claim 1, which recites the term "carbocycle." Regarding this term the present specification states: "an organic cyclic moiety in which the cyclic skeleton is comprised of only carbons." Specification at 29, lines 24-26; *see also* lines 31-32. This passage alone is evidence that the claim would reasonably apprise those skilled in the art that the scope of "carbocycle" includes both carbocyclic moieties containing a CH₂ group and a phenyl moiety. There is nothing inconsistent in limiting claim 2 or 10. Thus, the rejection is improper and should be reversed.

Furthermore, the rejection of claims 5 and 13 is improper and should be reversed. According to the Examiner, these claims are improper because the claims incorporate compounds by reference. However, it is well-established that applicants are their own lexicographers and may define in the claims what they regard as their invention essentially in whatever terms they choose so long as the terms are not used in ways that are contrary to

accepted meanings in the art. M.P.E.P. § 2173.01. Here, the explanation and evidence of record never even alleges that the terms are not used in ways that are contrary to accepted meanings in the art. Moreover, the Examiner knew what subject matter the claims were embracing. Faced with this evidence and explanation, how could one of ordinary skill in the art not be reasonably apprised of the claims scope? The rejection is improper and should be reversed.

B. The rejection of claims 1-2 and 9 under 35 U.S.C. § 103(a) over Bycroft *et al.*, Burbaum *et al.*, Gold *et al.*, and Hamilton should be reversed because evidence and explanation fail to make a prima facie case of obviousness.

The examiner improperly rejects claims 1-2 and 9 under 35 U.S.C. § 103(a) as obvious over Bycroft *et al.* (CA:84:106021), Burbaum *et al.*, (US 5,319,098) Gold *et al.* (US 4,818,749) and Hamilton (US 6,291,510) based on the structure of the claimed compounds alone. The evidence and explanation of record does not support the examiner's position. Accordingly, this rejection is procedurally improper and should be reversed.

According to the examiner, "the generic teaching rendered the claims obvious since it was explained supra the homologs, one methyl addition compounds, isosteric replacements, position isomers, which are structural prima facie would rendered the instant claims prima facie obvious and would not be repeated but incorporated by reference." Office Action dated May 30, 2003 (emphasis added). Yet even if, without admission, the examiner's statements were true, it would not establish a prima facie case of obviousness because there is no such thing as per se structural obviousness. M.P.E.P. § 2144.09. In other words, a prima facie obviousness cannot be established based on structure alone, regardless of whether the cited groups are homologs, positional isomers, or isosteres. Thus, the evidence and explanation of

record cannot support the examiner's position, the rejection is improper and should be reversed.

Furthermore, in the quoted text, the incorporation by reference is believed to refer to a statement made in relation to the rejection of claims under 35 U.S.C. § 112, second paragraph which states:

Due to the enormous amount of prior art both cited by the examiner and supplied on 26 pages of 1449 by applicants, individual rejections under 35 U.S.C. § 103(a) for such structural homologs, on methyl insertions, isosteric replacements, position isomers etc. will not be listed.

Office Action dated May 30, 2003 (emphasis added). So, in addition to Bycroft *et al.*, Burbaum *et al.*, Gold *et al.*, and Hamilton, the examiner cites an “enormous amount of prior art” which the examiner never identified. *Id.* Nor was the citation made after examining the references in accordance with MPEP § 609(2). As discussed above, *e.g.*, the examiner’s own notation indicates that the references submitted by Appellants on the form PTO-1449 received only a “cursory review as submitted.” *See* PTO-1449, *supra* (Appendix B).

In other words, the evidence and explanation never establish why the “enormous amount of prior art” would have rendered the present invention *prima facie* obvious. Thus, the indeterminate number of rejections are improper and must surely be reversed.

Finally, Bycroft *et al.* is a Chemical Abstract, not the publication named therein. It is not evidence of a prior invention or statutory bar as it is not necessarily an accurate summary of what is cited therein.

C. The rejection of claims 1-3, 5, 9-11 and 13 under 35 U.S.C. § 102(f) over WO 99/14998 should be reversed, because there was no prior invention "by another."

Claims 1-3, 5, 9-11, and 13 remain rejected under 35 U.S.C. § 102(f) over WO 99/14998. 35 U.S.C. § 102(f) states "A person shall be entitled to a patent unless...(f) he did not himself invent the subject matter sought to be patented." A rejection under § 102(f) is proper only if applicant "derived" the invention from another. M.P.E.P. § 2137 *citing Ex parte Kusko*, 215 USPQ 972, 974 (Bd. App. 1981). Derivation requires, *inter alia*, prior invention by another.

The examiner has provided no evidence supporting this rejection. As a preliminary matter, the Office has the initial burden of establishing anticipation. "[I]t is incumbent upon the Patent Office . . . to set forth clearly why it regards a claim to be anticipated. *In re Mullin*, 481 F.2d 1333, 1336, 179 U.S.P.Q. 97, 100 (C.C.P.A. 1973). For example, in *Mullin*, the court found the examiner's assertion that "Claims 1-5 are rejected as obviously anticipated by [a reference] under 35 U.S.C. 102" failed to inform the applicant why the claims are regarded as defective. *Id.* at 1336-37, 179 U.S.P.Q. at 100.

Here, the examiner argues, without much more, that claims 1-3, 5, and 9-11 are anticipated, because "WO 99/14998 has a US filing date prior to applicant's provisional date" and alleges that WO 99/14998 discloses compounds that "anticipate the claims." Office Action dated December 12, 2002. Nowhere in the record does the examiner indicate where in WO 99/14998 the allegedly anticipatory subject matter is located and why such disclosure is anticipatory. The examiner relies on the U.S. provisional application numbers 60/059,905 and 60/059,963 with filing dates of September 24, 1997, and September 25, 1997,

respectively, to which WO 99/14998 claims priority. However, the examiner does not provide any evidence that the allegedly anticipatory subject matter finds support those priority applications. The examiner neither cites to any disclosure in the priority documents nor even makes those documents of record and available to Appellants. As in *Mullin*, the Office has failed to inform Applicants why the claims were regarded as defective. Thus, the evidence and explanation fail to establish prior invention, and the rejection should be reversed.

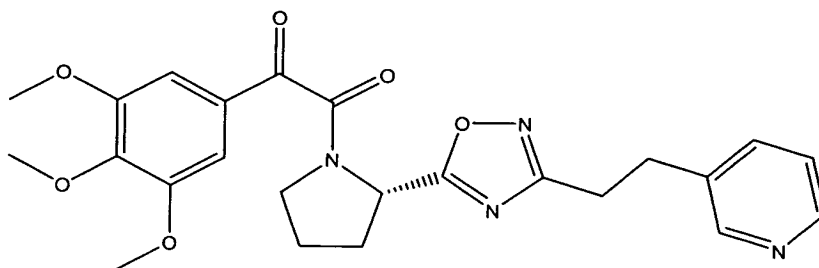
Because these documents were not made of record by the examiner, Appellants attach copies of priority application numbers 60/059,905 and 60/059,963 (Appendix C and D). Even though the examiner has not met the initial burden of establishing anticipation, Appellants nevertheless have reviewed application numbers 60/059,905 and 60/059,963 and find no support for cited compounds that anticipate claims 1-3, 5, 9-11, and 13. Accordingly, Appellants request reversal of this rejection.

D. The rejection of claims 1, 2, 6, 7, 9, and 10 under 35 U.S.C. 102(e), (f) or (g) as anticipated by Brumby (U.S. Patent No. 6,284,779) or Kato *et al.* (CA:135:226999) supplemented by Andersen *et al.* (CA:121:300841) should be withdrawn, because the evidence and explanation of record fail to support a prior invention "by another."

The evidence of record does not support a rejection of claims 1, 2, 6, 7, 9, and 10 under 35 U.S.C. 102(e), (f) or (g) as being anticipated by U.S. Patent No. 6,284,779 (Brumby *et al.*) or Kato *et al.*, *Chem. Abstracts* 135:226999, supplemented by Andersen *et al.*, *Chem. Abstracts* 121:300841. The examiner cites Chemical Abstracts which do not provide evidence of critical date of the document. As such, Appellants are unable to determine whether the cited documents are, in fact, prior art. Additionally, because the examiner cites the Chemical Abstract, which is a summary of published document, it is not established on

the record that the disclosure in the Chemical Abstract accurately reflects the disclosure of the underlying published document. Accordingly, there is no evidence of record that the claims on appeal are anticipated.

The examiner also alleges that U.S. Patent No. 6,284,779 anticipates claims 1, 2, 6, 7, 9, and 10, but the evidence and explanation of record do not establish that '779 patent reads on the instant claims. ChemDraw[®] Ultra (Version 8.0, April 23, 2003, CambridgeSoft) indicates 3-(2-(3-pyridyl)ethyl)-5-[(2s)-1-(3,4,5-trimethoxyphenylglyoxyloyl)-pyrrolidin-2-yl]1,2,4-oxadiazole, the formula cited by the examiner at column 12, example 2 of the 779 patent, has the following structure:



It is not Appellants' burden to search for the evidence supporting an anticipation rejection. A factual finding that is material to patentability can neither stand if it is supported only by conclusory statements nor be resolved on the subjective belief of an examiner. *In re Lee*, 277 F.3d 1338, 1346, 61 USPQ2d 1430, 1433 (Fed. Cir. 2002). Because the cited references do not provide the evidence necessary establish prior invention by another, the rejection is improper and should be reversed.

- E. The rejection of claims 1-3 and 9-11 under 35 U.S.C. 103(a) over U.S. Patent Nos. 5,859,031, 5,945,441, 6,177,455 and 6,291,510 in view of Silverman or Bungaard, further in view of U.S. Patent Nos. 5,801,187 and 6,218,544 should be reversed, because the evidence and explanation fail to make a prima facie case of obviousness.**

The evidence and explanation of record do not establish a prima facie case of obviousness. Claims 1-3 and 9-11 stand rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent Nos. 5,859,031, 5,945,441, 6,177,455 and 6,291,510 in view of Silverman, THE ORGANIC CHEMISTRY OF DRUG DESIGN AND DRUG ACTION, Academic Press, 1993, pp. 354-357), or Bungaard, DESIGN OF PRODRUGS, Elsevier, 1985, pp. 3-4, further in view of U.S. Patent Nos. 5,801,187 and 6,218,544. To establish a *prima facie* case of obviousness under 35 U.S.C. § 103, the U.S.P.T.O must meet three basic elements:

First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

M.P.E.P. § 2142.

In support of the original rejection the examiner contended:

Hamilton '031, '441, '445, '510 disclosed pyrrolidinyl compounds with 1-dioxolyl substitution and 2-carboxylic ester and their compositions which have identical utility as the instantly claims i.e. neurotrophic/immunophilin inhibitors.

Office Action dated December 12, 2002. The examiner continues stating:

No evidence in the record indicated that the esters of Hamilton '031, '441, '445, '510 should not function in physiological conditions according to common knowledge of the art as taught by Bungaard or Silverman.

Id.

An obviousness analysis under section 103 requires, *inter alia*, consideration of whether the prior art also would have revealed that in making the claimed composition of device, or in carrying out the claimed process those of ordinary skill would have a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991) citing *In Re Dow Chemical*, 837 F.2d 469, 473 (Fed. Cir. 1988). "Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *Id.*

The examiner misapplies the legal standards to be applied in an obviousness determination. When considering whether there is motivation to modify or combine a reference teaching, the relevant inquiry is not whether reference teaching can be combined. M.P.E.P. § 2143.01. The relevant inquiry is whether one of skill in the art at the time of filing would find an explicit or implicit suggestion, contained within the references themselves, to modify or combine each reference's teachings to make the claimed invention. M.P.E.P. § 2143.01. Importantly, with respect to this motivation, the Federal Circuit places the burden on the Office to present "clear and particular" evidence showing motivation to combine. *In re Dembiczak*, 50 USPQ2d 1614 (Fed. Cir. 1999). *In re Dembiczak*, 50 USPQ2d 1614 (Fed. Cir. 1999).

Here, nevertheless, such clear and particular evidence is conspicuously absent from the record. Even if the examiner argues that because the invention works, there existed, at the time of filing, a motivation to modify the teachings of claims 1, 4 and 18 of U.S. Patent No. 5,859,031 and claim 4 of U.S. Patent No. 6,291,510 in view of Silverman, *supra*, or Bungaard, *supra*, further in view of claims 1-8 of U.S. Patent No. 5,801,187 and claim 1 of U.S. Patent No. 6,218,544 to arrive at the invention of claims 1-3 and 9-11, the rejection

cannot be made proper by merely stating how the teachings can be combined. Since there is no explanation and evidence of a reason to combine, the examiner must be using improper hindsight reconstruction in asserting that the cited art provided the requisite motivation to use the esters of U.S. Patent Nos. 5,859,031 and US 6,291,510 as prodrugs, the efficacy of which is dependent upon proper cleavage to yield an effective free acid form. *See In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988), *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991).

Similarly, when ascertaining whether there was a reasonable expectation of success that the claimed invention would be effective for its intended purpose, the relevant inquiry is whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would work at the time of filing. The relevant inquiry is not whether the claimed invention was effective. Like the motivation to combine, the reasonable expectation of success must be found in prior art, not in the applicant's disclosure. *Vaeck*, 947 F.2d at 493. Here, however, the examiner again argues that because esters can be hydrolyzed *in vivo* this provides the requisite reasonable expectation of success. Not only is the examiner's argument flawed and against the teaching of the Federal Circuit case law, it is not based on the teachings of record, which are inconsistent with the examiner's position.

Specifically, the cited documents actually contradict the examiner's position. "Some prodrugs are not designed as such; biotransformations are fortuitous, and it is discovered after isolation and testing of the metabolites that activation of the drug had occurred." Silverman, *supra* at 354. Furthermore, both Bungaard, *supra*, and Silverman, *supra*, acknowledge that some esters are ineffective as prodrugs. "Sometimes, simple aliphatic or aromatic esters may not be sufficiently labile *in vivo* to ensure a sufficiently high rate and extent of prodrug

conversion.” Bungaard, *supra* at 4. “[I]n some cases the esters are not very good substrates for the endogenous esterases, sulfatases, or phosphatases, and they may not be hydrolyzed at a rapid enough rate.” Silverman, *supra*, at page 356.

As a result, even if some, but not all, esters may be effective as prodrugs, and the ‘031, ‘441, ‘445, ‘510 patents recite esters, these premises do not allow one to validly conclude that the esters of the ‘031, ‘441, ‘445, ‘510 patents are effective prodrugs. Indeed, that argument has the same form as: some cars are black; the Pinto is a car; thus, the Pinto is black. Such an argument would be logically invalid and flawed.

The explanation and evidence of record lack clear and particular evidence of a motivation to combine and a reasonable expectation of success. In other words, the examiner failed to establish a *prima facie* case. The rejection is improper and should be reversed.

F. The rejection of claims 1-3 and 9-11 under the judicially created doctrine of obviousness-type double patenting over the claims of U.S. Patent Nos. 5,859,031, 5,945,441, 6,177,455 and 6,291,510 in view of Silverman or Bungaard further in view of U.S. Patent Nos. 5,801,187 and 6,218,544 should be reversed, because the evidence and explanation fail to make a *prima facie* case of obviousness has not been established.

The evidence and explanation of record fails to support the rejection of claims 1-3 and 9-11 as being unpatentable over claims 1, 4 and 18 of U.S. Patent No. 5,859,031 and claim 4 of U.S. Patent No. 6,291,510 in view of Silverman or Bungaard further in view of claims 1-8 of U.S. Patent No. 5,801,187 and claim 1 of U.S. Patent No. 6,218,544.

Determination of obviousness-type double patenting essentially involves the determination of obviousness under 35 U.S.C. § 103. *In re Longi*, 759 F.2d 887, 892 n.4, 225 U.S.P.Q. 645, 648 n.4 (Fed. Cir. 1985). One, however, considers not the entire patent that

forms the primary basis of the rejection but only its claims. For the same reasons discussed with regard to the rejection under section 103 of these same claims over these same references ("issue E"), the examiner fails to establish a prima facie case of obviousness.

In support of the original rejection the examiner contended:

Hamilton '031...[and] '510 disclosed all the elements of the claims except the compounds are in form of an ester of the instant carboxylic acid or carboxylic acid isostere. Bungaard taught that ester is a "prodrug" of free carboxylic acid compounds since naturally, under physiological conditions, the ester will be hydrolyzed to acid by esterases which can be found in the blood, liver and other organs or tissues (see Bungaard p. 3-4 para bridging) while Silverman taught the same concept further taught the same approach can be extend to sulfate or phosphate esters (see p. 356) and the modification of the ester moiety to increase the hydrolyzation rate (see p. 357).

Office Action dated December 12, 2002. The examiner further contends:

the Silverman or Bungard references disclosed that esters will be hydrolyzed in vivo because of the abundant existence of liver, blood or organ esterase. Therefore, esters *will be hydrolyzed in vivo* is factual not mere suggestion. Since hydrolysis is factual, it will happen, the free acids will exist in vivo. Applicants then presented a self conflicting argument by alleging unpredictability of the activity of the free acid. The question of whether hydrolyzed ester i.e. free acid, will have activity is unpredictable will face conflicting issues. If applicants are alleging that the free acid does "not" having activity, then, the instant claims must face a 101 or 112 first paragraph issue.

Office Action dated May 30, 2003.

This obviousness-type double patenting rejection suffers from the same defects as the obviousness rejection of claims 1-3 and 9-11 which was addressing in issue "E". Both rejections lack any explanation and evidence of record to provide clear and particular

motivation to combine and a reasonable expectation of success. Here again, the examiner failed to establish a prima facie case of obviousness. The rejection should be reversed.

G. The refusal to examine the entire scope of the claims is an improper rejection and should be reversed.

The examiner's refusal to examine the entire scope of the claims is an effective withdrawal of subject matter from consideration tantamount to a rejection of the claims. *In re Haas*, 198 USPQ 334 (CCPA 1978), citing *In re Haas* 179 USPQ 623 (CCPA 1973). The proper scope of examination of the claims on appeal includes compounds having both a pyrrolidinyl core and a heterocyclic-pyrrolidinyl core.

The application-as-filed was subjected to a fourteen-way restriction. Office Action dated September 16, 2003. Each group further required a species election. *Id.* Appellants provisionally elected Group I, containing claims 1-5 and 8-13, with traverse. Response to Restriction Requirement, dated October 16, 2002. The examiner alleges that the subject matter to be examined includes only compounds where $n=1$ and R^2 is a heterocycle. The examiner further states that Appellants did not provide evidence that the heterocyclic-pyrrolidinyl core "is of the same core as pyrrolidinyl only core." Office Action dated May 30, 2003 at page 2. As such, examiner alleges that the current form of the claims are drawn to non-elected subject matter.

Upon a determination that the elected species is novel and nonobvious, the Examiner is required to expand the scope of examination beyond that of the elected species to include all the currently claimed subject matter. The M.P.E.P. instructs that when species election is required in a Markush-type generic claim, the species will be examined. M.P.E.P. § 803.02.

Upon examination of the species, if no prior art is "found that anticipates or renders obvious the elected species, the search of the Markush-type claim will be extended." M.P.E.P. § 803.02. Therefore, upon determination of patentability, Appellants are entitled to a broadened scope of examination which includes both a pyrrolidinyl core and a heterocyclic-pyrrolidinyl core.

Although the examiner has not expressly stated that subject matter other than compounds where $n=1$ and R^2 is a heterocycle are withdrawn from consideration, refusing to examine the entire scope of the claims is effectively withdrawal of the claims from consideration. Such withdrawal is a rejection that is reviewable by the board. *In re Haas*, 198 USPQ 334 (CCPA 1978), citing *In re Haas* 179 USPQ 623 (CCPA 1973). Appellants request reversal of this rejection.

APPENDICES

Appendix A contains a copy of the claims involved in the appeal.

Appendix B contains a copy of U.S.P.T.O. form PTO-1449 considered by the examiner on December 5, 2002.

Appendix C contains a copy of U.S. application number 60/059,905.

Appendix D contains a copy of U.S. application number 60/059,963.

CONCLUSION

For the reasons given above, the Board of Patent Appeals and Interferences
should reverse each rejection.

Respectfully submitted,

Date DECEMBER 02, 2003

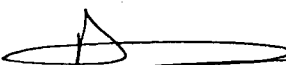
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By



Amy M. Rocklin, Reg. No. 47,033

for

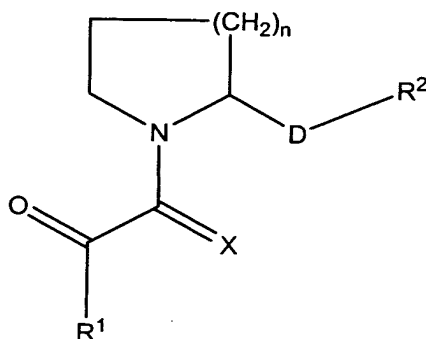
Suet M. Chong

Attorney for Applicant

Registration No. 38,104

APPENDIX A

1. A compound



or a pharmaceutically acceptable salt, ester, or solvate of the compound, wherein:

n is 1;

X is either O or S;

R¹ is C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, aryl, heteroaryl, carbocycle, or heterocycle;

D is a bond, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ alkenyl or C₂-C₁₀ alkynyl;

R² is carboxylic acid or a carboxylic acid isostere;

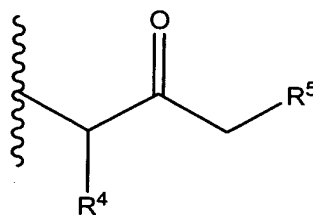
said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, or carboxylic acid isostere is optionally substituted with one or more substituents selected from R³ and Z;

R³ and Z are independently hydrogen, hydroxy, halo, haloalkyl, thiocarbonyl, alkoxy, alkenoxy, alkylaryloxy, aryloxy, arylalkyloxy, cyano, nitro, imino, alkylamino, aminoalkyl, sulfhydryl, thioalkyl, alkylthio, sulfonyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl or alkynyl, aryl, aralkyl, heteroaryl, carbocycle, heterocycle, or CO₂R⁷;

R⁷ is hydrogen, C₁-C₉ straight or branched chain alkyl or C₂-C₉ straight or branched chain alkenyl;

provided that when D is a bond and R^2 is COOH, then R^1 is not C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl, C_5 - C_7 cycloalkyl, C_5 - C_7 cycloalkenyl, phenylamine, 2-(3, 4-dichlorophenyl)ethyl, hydroxy, ethoxy, benzyl, or Ar^1 , wherein Ar^1 is 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 1-pyridyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or phenyl, and wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl, or Ar^1 is optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, nitro, trifluoromethyl, C_1 - C_9 straight or branched alkyl, C_2 - C_9 straight or branched alkenyl, C_1 - C_4 alkoxy, C_2 - C_4 alkenyloxy, phenoxy, benzyloxy, COOH, and amino;

further provided that when D is a bond, R^2 is the carboxylic acid isostere $-CONZ(R^3)$, Z is hydrogen or C_1 - C_6 alkyl, and R^3 is phenyl, or C_2 - C_6 straight or branched chain alkyl or alkenyl, wherein said alkyl is unsubstituted or substituted in one or more positions with Ar^2 as defined below, C_3 - C_8 cycloalkyl, cycloalkyl connected by methyl or a C_2 - C_6 straight or branched chain alkyl or alkenyl, C_1 - C_4 alkyl ester, or Ar^3 wherein Ar^3 is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or phenyl, having one to three substituents independently selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched alkyl, C_2 - C_6 straight or branched alkenyl, C_1 - C_4 alkoxy, C_2 - C_4 alkenyloxy, phenoxy, benzyloxy, and amino; wherein said alkyl ester is optionally substituted with phenyl; or R^3 is the fragment:



wherein R⁴ is selected from the group consisting of straight or branched chain C₁-C₈ alkyl optionally substituted with C₃-C₈ cycloalkyl, benzyl, or Ar² as defined below, and wherein R² is COOZ or CONR⁶, wherein R⁶ is selected from the group consisting of hydrogen, C₁-C₆ straight or branched alkyl, and C₂-C₆ straight or branched alkenyl, and wherein R⁵ is selected from the group consisting of phenyl, benzyl, C₁-C₆ straight or branched alkyl, and C₂-C₆ straight or branched alkenyl, wherein said alkyl or alkenyl is optionally substituted with phenyl; then R¹ is not C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, substituted thiophene, or C₁-C₄ alkoxy, wherein said alkyl or alkenyl is optionally substituted in one or more positions with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, or Ar² as defined below, wherein said alkyl, alkenyl, cycloalkyl or cycloalkenyl is optionally substituted with C₁-C₄ alkyl, C₂-C₄ alkenyl, or hydroxy, and wherein Ar² is 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or phenyl, having one to three substituents selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched alkyl, C₂-C₆ straight or branched alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, and amino;

further provided that when X is O, D is a bond, and R² is -CONH₂, then R¹ is not methyl, ethyl, iso-propyl, iso-butyl, iso-pentyl, 4-methylpentyl, indolyl, phenyl, or hydroxyphenyl;

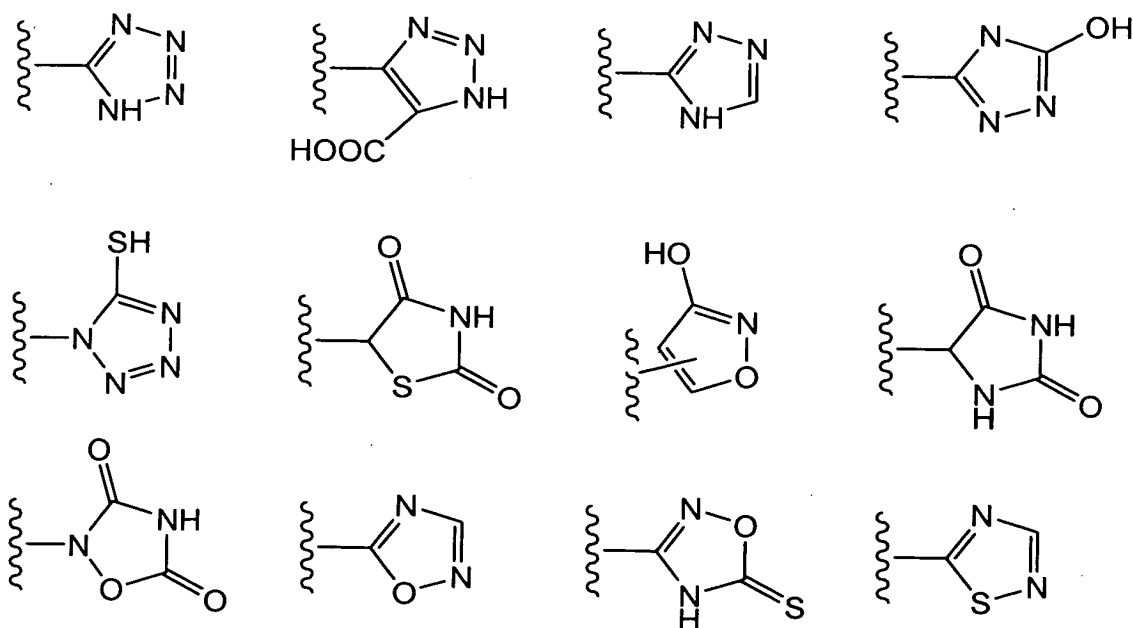
further provided that when X is O, D is a bond, and R² is cyano, then R¹ is not methyl;

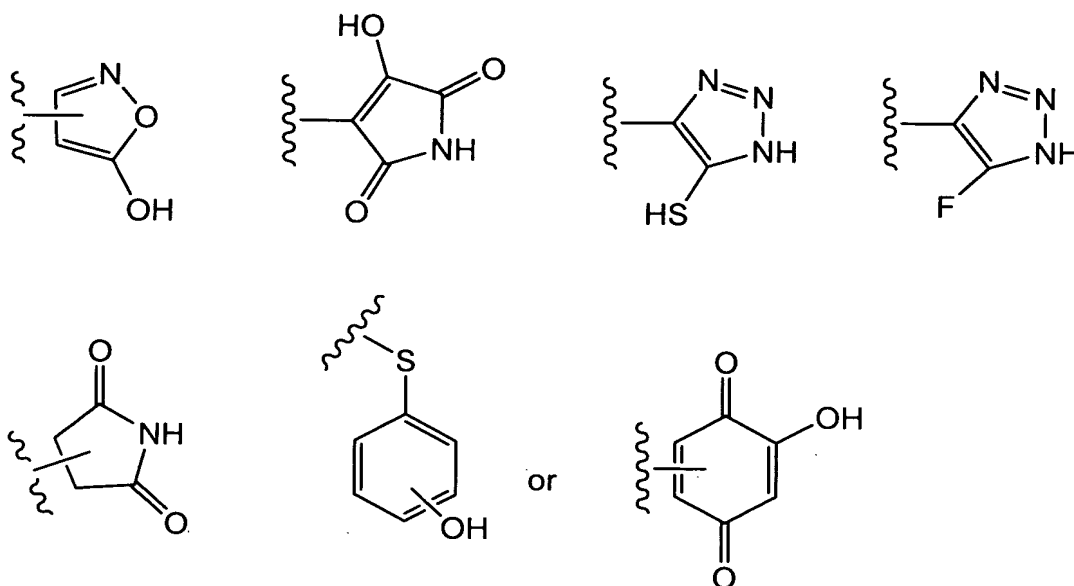
further provided that : when D is CH₂, then R² is not -OMe, -NHMe, or substituted -NHcyclohexyl; and

further provided that when D is CH₂, and R² is -OH, then R¹ is not phenyl or pyrrolidinemethanol.

2. The compound of claim 1, wherein R² is a carbocycle or heterocycle containing any combination of CH², O, S, or N in any chemically stable oxidation state, wherein any of the atoms of said ring structure is optionally substituted in one or more positions with R³.

3. The compound of claim 1, wherein R² is





wherein the atoms of said ring structure is optionally substituted at one or more positions with R^3 .

4. (canceled)

5. The compounds, (2S)-1-(1,2-dioxo-3,3—dimethylpentyl)-2-hydroxymethylpyrrolidine; (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinetetrazole; (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarbonitrile; and compounds 1, 3, 5, 8, 11, 14, 17, 21, 24-32, 34, 38-40, 44, 45, 47-52, 62, 64-68, 73-98, 101, 102, 106, 108-117 and 119-137 of Tables I, II, and III.

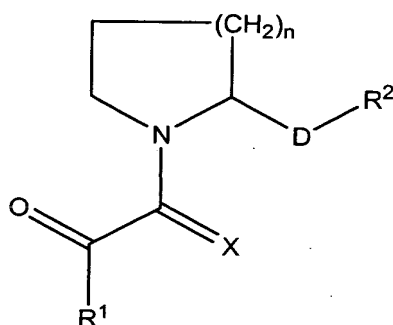
6. The compound 1-{2-[3-(4-Fluorophenyl)(1,2,4-oxadiazol-5-yl)] pyrrolidinyl}-3,3-di-methylpentane-1,2-dione.

7. The compound 3,3-Dimethyl-1-[2-(3-methyl(1,2,4-oxadiazol-5-yl))pyrrolidinyl]pentane-1, 2-dione.

8. (canceled)

9. A pharmaceutical composition comprising:

(i) a compound of formula I



or a pharmaceutically acceptable salt, ester, or solvate of the compound, wherein:

n is 1;

X is either O or S;

R¹ is C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, aryl, heteroaryl, carbocycle, or heterocycle;

D is a bond, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ alkenyl or C₂-C₁₀ alkynyl;

R² is carboxylic acid or a carboxylic acid isostere;

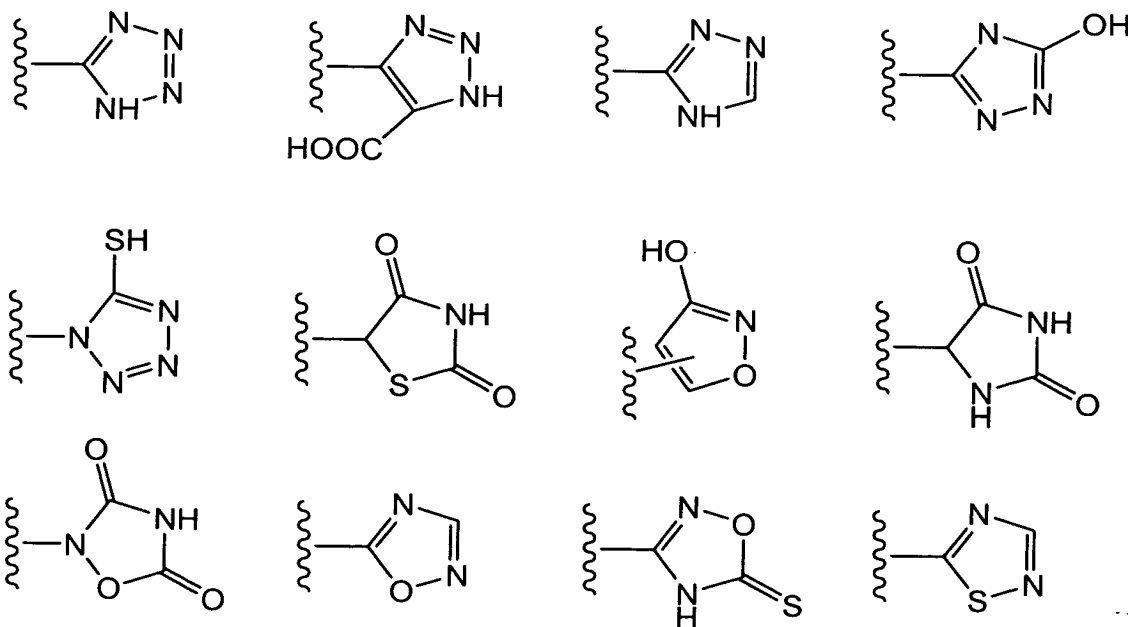
said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, or heterocycle is optionally substituted with one or more substituents selected from hydroxy, halo, haloalkyl, thiocarbonyl, alkoxy, alkenoxy, alkylaryloxy, aryloxy, arylalkyloxy, cyano, nitro, imino,

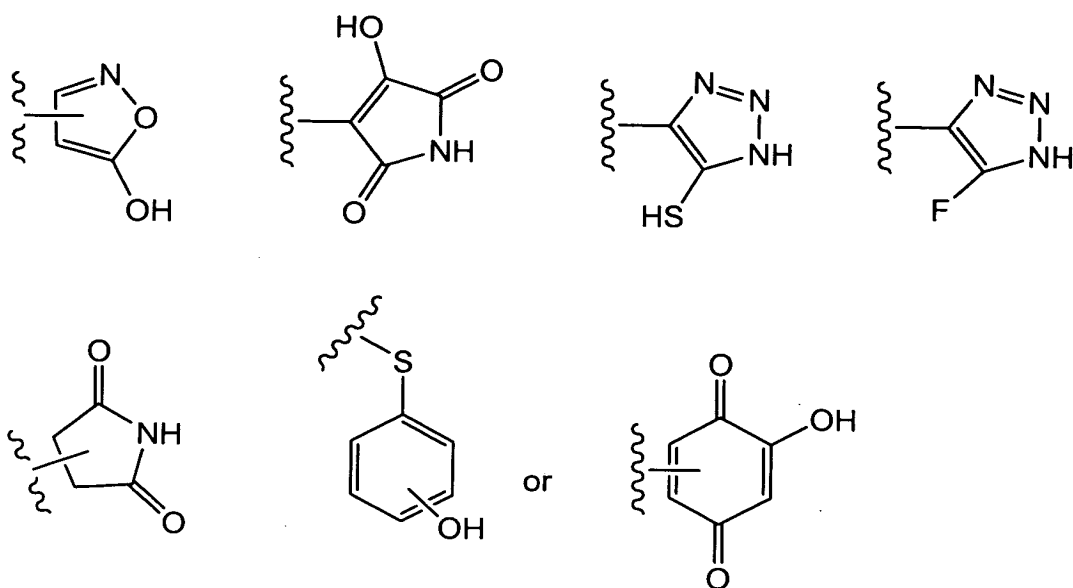
alkylamino, aminoalkyl, sulfhydryl, thioalkyl, alkylthio, sulfonyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl or alkynyl, aryl, aralkyl, heteroaryl, carbocycle, heterocycle, and CO₂R⁷ wherein R⁷ is hydrogen, C₁-C₉ straight or branched chain alkyl or C₂-C₉ straight or branched chain alkenyl; and

(ii) a pharmaceutically acceptable carrier.

10. The pharmaceutical composition of claim 9, wherein R² is a carbocycle or heterocycle containing any combination of CH₂, O, S, or N in any chemically stable oxidation state, wherein any of the atoms of said ring structure is optionally substituted in one or more positions with R³.

11. The pharmaceutical composition of claim 9, wherein R² is:





wherein the atoms of said ring structure is optionally substituted at one or more positions with R^3 .

12. (canceled)

13. The pharmaceutical composition of claim 9, wherein the compound is selected from the group consisting of compounds 1, 3, 5, 8, 11, 14, 17, 21, 24-32, 34, 38-40, 44, 45, 47-52, 62, 64-68, 73-98, 101, 102, 106, 108-117 and 119-137 of Tables I, II and III.

14-82. (canceled)

APPENDIX B

LIST OF PATENTS AND OTHER ITEMS FOR APPLICANT'S
INFORMATION DISCLOSURE STATEMENT

APPLICANT:

Gregory S. HAMILTON et al.

FILING DATE:

August 2, 2001

GROUP:

1614

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

EXAMINER INITIAL	DOCUMENT NUMBER	DATE	NAME	CLASS	SUB CLASS	FILING DATE
AA						
AB						
AC						
AD						
AE						
AF						
AG						
AH						

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB CLASS	TRANSLATION YES NO
AI	WO 94/07858 A	9/27/93				Yes
AJ	EP 564 924	10/13/93				Yes

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

AK	Australian Patent Office Search Report
AL	Chemical Abstracts Registry Number 76391-12-3
AM	Chemical Abstracts Registry Number 83079-95-2
AN	Chemical Abstracts Registry Number 83079-96-3
AO	Chemical Abstracts Registry Number 53935-75-4
AP	T. Kitazaki et al., "Synthesis and Human Immunodeficiency Virus (HIV-1) Protease Inhibitory Activity of Tripeptide Analogues Containing a Dioxoethylene Moiety", Chem. Pharm. Bull., 1994, Vol. 42, pp. 2636-2460.

C-11202.1

EXAMINER:

Not yet assigned

DATE CONSIDERED:

12/5/02

EXAMINER: Initial if reference is considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include a copy of this form with next communication to applicant



FORM PTO-1449

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Attorney Docket
AR762-XXASerial Number
09/920,017

Applicant

HAMILTON et al.

Filing Date

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Group Art Unit

1614

U.S. PATENT DOCUMENTS

Examiner Initial	Document Number	Date	Name	Class	Sub-Class	Filing Date
	AA 5,646,167	7/8/97	MacPherson et al.			6/7/95
	AB 5,840,736	11/24/98	VERTEX			
	AC 5,811,434	09/22/98	VERTEX			

FOREIGN PATENT DOCUMENTS

Document Number	Date	Country	Class	Sub-Class	Translation
AD WO 98/29117	09/07/98	PCT			Yes
AE WO 98/24805	11/06/98	PCT			Yes
AF WO 98/22432	28/05/98	PCT			No
AG WO 98/20893	22/05/98	PCT			Yes
AH WO 98/20892	22/05/98	PCT			Yes
AI WO 98/20891	22/05/98	PCT			Yes
AJ WO 98/13343	02/04/98	PCT			Yes
AK WO 98/08827	05/03/98	PCT			Yes
AL WO 97/38008	16/10/97	PCT			Yes
AM WO 97/36869	09/10/97	PCT			Yes
AN WO 97/31898	04/09/97	PCT			Yes
AO WO 97/23458	03/07/97	PCT			Yes
AP WO 97/23202	03/07/97	PCT			Yes
AQ WO 96/41609	27/12/96	PCT			Yes
AR WO 96/40140	19/12/96	PCT			Yes
AS WO 96/36630	21/11/96	PCT			Yes
AT WO 96/20949	11/07/96	PCT			Yes
AU WO 96/20725	11/07/96	PCT			Yes
AV WO 96/17816	13/06/96	PCT			Yes
AW WO 96/15101	23/05/96	PCT			Yes
AX WO 95/35367	28/12/95	PCT			Yes
AY WO 95/35308	28/12/95	PCT			Yes
AZ WO 95/34303	21/12/95	PCT			Yes

Examiner

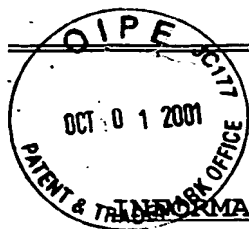
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FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Sub-Class	Translation
	BA	WO 95/12572	11/05/95	PCT			Yes
	BB	WO 95/12398	11/05/95	PCT			Yes
	BC	WO 95/02684	26/01/95	PCT			Yes
	BD	WO 94/15900	21/07/94	PCT			Yes
	BE	WO 94/14428	07/07/94	PCT			Yes
	BF	WO 94/13629	23/06/94	PCT			Yes
	BG	WO 94/07858	14/04/94	PCT			Yes
	BH	WO 94/04129	03/03/94	PCT			Yes
	BI	WO 94/03476	17/02/94	PCT			Yes
	BJ	WO 93/18736	30/09/93	PCT			Yes
	BK	WO 93/14762	05/08/93	PCT			Yes
	BL	WO 93/14072	22/07/93	PCT			Yes
	BM	WO 93/13066	08/07/93	PCT			Yes
	BN	WO 92/21313	10/12/92	PCT			Yes
	BO	WO 92/19745	12/11/92	PCT			Yes
	BP	WO 92/19593	12/11/92	PCT			Yes
	BQ	WO 92/11850	23/07/92	PCT			Yes
	BR	WO 92/11245	09/07/92	PCT			Yes
	BS	WO 92/04370	19/03/92	PCT			Yes
	BT	WO 91/13088	05/09/91	PCT			Yes
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	BV	WO 89/06234	13/07/89	PCT			Yes
	BW	WO 88/09789	15/12/88	PCT			Yes
	BX	WO 88/00040	14/01/88	PCT			Yes
	BY	WO 85/04577	24/10/85	PCT			Yes
	BZ	EP 823419	11/02/98	EP			Yes
	BAA	EP 652229	10/05/95	EP			Yes
	BAB	EP 610744	13/10/93	EP			Yes
	BAC	EP 519819	23/12/92	EP			No

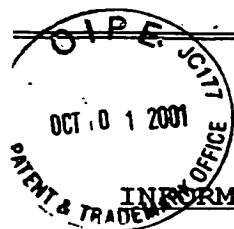
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1614

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Sub-Class	Translation
	CA	EP 494005	08/07/92	EP			No
	CB	EP 471135	19/02/92	EP			Yes
	CC	EP 468339	29/01/92	EP			Yes
	CD	EP 476933	25/03/92	EP			Yes
	CE	EP 443983	28/08/91	EP			No
	CF	EP 405994	02/01/91	EP			Yes
	CG	EP 378318	18/07/90	EP			Yes
	CH	EP 352000	24/01/90	EP			Yes
	CI	EP 333174	20/09/89	EP			Yes
	CJ	EP 196841	08/10/86	EP			Yes
	CK	EP 088350	14/09/83	EP			Yes
	CL	EP 073143	02/03/83	EP			Yes
	CM	EP 050800	05/05/82	EP			Yes
	CN	EP 048159	24/03/82	EP			Yes
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	CR	DE 3508251	11/09/86	DE			No
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	CT	JP 05-194235		JP			No
	CU	JP 05-178824		JP			No
	CV	ZA 9207782	09/10/92	ZA			Yes

OTHER (Including Author, Title, Date, Pertinent Pages, etc.)

CW

Andrus, Merrit B., "Structure-based design of an acyclic ligand that bridges FKBP 12 and calcineurin," J. Am. Chem. Soc., 1993, 115(2), 10420-21.

Examiner

Date Considered

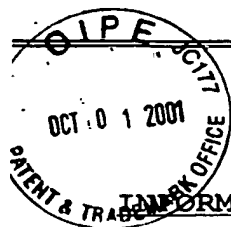
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HAMILTON et al.Filing Date
August 2, 2001Group Art Unit
1614

OTHER (Including Author, Title, Date, Pertinent Pages, etc.)

CC/cursory review as submitted	DA	Armistead, D.M. et al., "Design, synthesis and structure of non-macrocyclic inhibitors of FKBP12, the major binding protein for the immunosuppressant FK506," <u>Acta Crystallogr.</u> , 1995, D51(4), 522-8.
	DB	Askin, D. et al., "Chemistry of FK-506: benzilic acid rearrangement of the tricarbonyl system," <u>Tetrahedron Lett.</u> , 1989, 30(6), 671-74.
	DC	Baader, Ekkehard et al., "Inhibition of prolyl 4-hydroxylase by oxalyl amino acid derivatives in vitro, in isolated microsomes and in embryonic chicken tissues," <u>Biochem. J.</u> , 1994, 300(2), 525-30.
	DD	Baumann, K. et al., "Synthesis and oxidative cleavage of the major equilibrium products of ascomycin and FK506," <u>Tetrahedron Lett.</u> , 1995, 26(13), 2231-34.
	DE	Bender, D. et al., "Periodate Oxidation of α -Keto γ -Lactams. Enol Oxidation and β -Lactam Formation Mechanism of Periodate Hydroxylation Reactions," <u>J. Org. Chem.</u> , 1978, 43, 3354-62.
	DF	Birkenshaw, T. et al., "Synthetic FKBP12 Ligands Design and Synthesis of Pyranose Replacements," <u>Bioorg. Med. Chem. Lett.</u> , 1994, 4, 2501-06.
	DG	Boulmedais, A. et al., "Stereochimie de la reduction electrochimique d'acetoamides optiquement actives Electroreduction de benzoylforamides derives de la S(-)-proline," <u>Bull. Soc. Chim. Fr.</u> , 1988, 185-91.
	DH	Bycroft, B. et al., "Efficient Asymmetric Synthesis of α -Amino Acids from α -Keto Acids and Ammonia with Conservation of the Chiral Reagent," <u>J.C.S. Chem. Comm.</u> , 1975, 988-89.
	DI	Caffrey, M. et al., "Synthesis and Evaluation of Dual Domain Macrocyclic FKBP12 Ligands," <u>Bioorg. Med. Chem. Lett.</u> , 1994, 4, 2507-10.
	DJ	Cai, D. and Still, W.C., "Synthesis of the α , β -Diketo Amide Segment of the Novel Immunosuppressant FK506," <u>J. Org. Chem.</u> , 1988, 53, 4643-44.

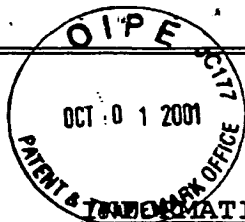
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HAMILTON et al.Filing Date
August 2, 2001Group Art Unit
1614

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EA

Callens, Roland E.A. et al., "Preparation of Trans-5-Hydroxy-L-Pipecolic Acid and Cis-4-Hydroxy-L-Pipecolic Acid from L-Baikiaia (1,2,5,6-L-Tetrahydropyridine-2-Carboxylic Acid)," Bull. Soc. Chim. Belg., 1982, 91, 713-23.

EB

Caufield, C. and Musser, J., "Macrocyclic Immunomodulators," Ann. Rep. Med. Chem., 1989, 195-204

EC

Chakaraborty, Tushar K. et al., "Design and Synthesis of a rapamycin-based high affinity binding FKBP12 Ligand," Chem. Biol., 1995, 2(3), 157-61.

ED

Chakaraborty, Tushar K., "Studies Directed Towards the Development of Cyclic Peptide Based Analogs of Macrolide Immunosuppressants," Pure Appl. Chem., 1996, 68(3), 565-68.

EE

Coleman, R. and Danishefsky, S., "Degradation and Manipulations of the Immunosuppressant FK506: Preparation of Potential Synthetic Intermediates," Heterocycles, 1989, 28, 157-61.

EF

Colombo, L. et al., "Enantioselective Synthesis of Secondary Alcohols in the Presence of Chiral Ligands," Tetrahedron, 1982, 38(17), 2725-27.

EG

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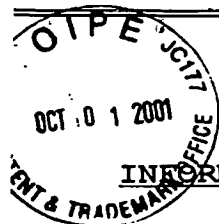
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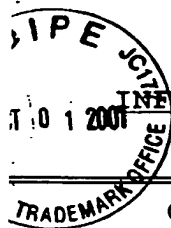
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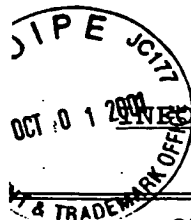
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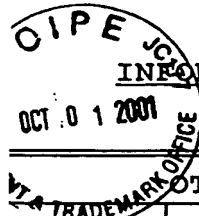
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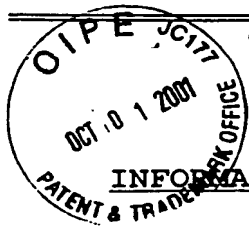
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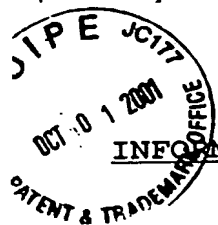
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BH	Gudasheva, T.A. et al., "Synthesis and anti-amnesic activity of a series of N-acylprolyl-containing dipeptides," <u>Eur. J. Med. Chem.</u> , 31 (1996), 151-7.
BI	Waldmann, H., "Proline Benzyl Ester as Chiral Auxiliary in Barbier-Type Reactions in Aqueous Solution," <u>Synlett</u> , 1990, 627-8.
BJ	Munegumi, T. et al., "Diastereoselective Catalytic Hydrogenation of N ^α -Pyruvoyl-(S)-prolinamide," <u>Bull. Chem. Soc. Jpn.</u> , 63 (1990)1832-34.
BK	Hausler, J. et al., "Hydroxylsubstituierie Cyclo-dipeptide Durch Ringschluff Von Pyruvoylaminosaure-amiden," <u>Chem. Ber.</u> , 107 (1974) 2804-15.
BL	Steglich, W. et al., "Eine rationelle Synthese von N-Trifluoroacetylaminosauren," <u>Synthesis</u> , (1976) 399-401.

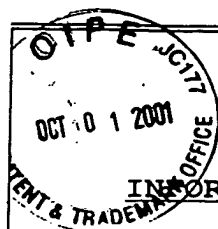
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Attorney Docket
AR762-XXASerial Number
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August 2, 2001Group Art Unit
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Examiner Initial		Document Number	Date	Name	Class	Sub-Class	Filing Date
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	AW	WO 99/45006	09/10/98	PCT			Yes
	AX						

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August 2, 2001Group Art Unit
1614**U.S. PATENT DOCUMENTS**

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	AJ	WO 9962881	12/09/99	PCT			Yes
	AK	PCT/US99/28663	11/24/00	PCT/ISA/220			Yes
	AJ	PCT/US99/28663	12/12/00	PCT IPEA/408			Yes
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	AJ	WO 9855091	12/10/98	PCT			Yes
	AK	WO 9855090	12/10/98	PCT			Yes
	AJ	WO 9837885	9/3/98	PCT			Yes
	AM	WO 9731898	9/4/97	PCT			Yes
	AN	WO 9640633	12/19/96	PCT			Yes
	AO	WO 9606097	2/29/96	PCT			Yes
	AP	WO 9200278	1/9/92	PCT			Yes

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Examiner Initial	Document Number	Date	Name	Class	Sub-Class	Filing Date
	AA	5,721,256	2/24/98	Hamilton et al.		2/12/97
	AB	5,717,092	2/10/98	Armistead et al.		3/29/96
	AC	5,714,510	2/3/98	Proctor		6/5/95
	AD	5,703,088	12/30/97	Sharpe et al.		6/4/92
	AE	5,696,135	12/9/97	Steiner et al.		5/28/96
	AF	5,654,332	8/5/97	Armistead et al.		6/8/95
	AG	5,620,971	4/15/97	Armistead et al.		3/25/94
	AH	5,614,547	3/25/97	Hamilton et al.		6/7/95
	AI	5,604,294	2/18/97	Luly et al.		3/14/94
	AJ	5,599,927	2/4/97	Or et al.		12/15/95
	AK	5,585,397	12/17/96	Tung et al.		3/17/94
	AL	5,543,423	8/6/96	Zelle et al.		1/23/95
	AM	5,541,192	7/30/96	Skotnicki et al.		5/24/95
	AN	5,541,189	7/30/96	Luly et al.		4/18/95
	AO	5,530,121	6/25/96	Kao et al.		5/25/95
	AP	5,527,907	6/18/96	Or et al.		10/26/94
	AQ	5,516,797	5/14/96	Armistead et al.		4/11/94
	AR	5,506,243	4/9/96	Ando et al.		4/28/94
	AS	5,504,197	4/2/96	Schubert et al.		3/22/93
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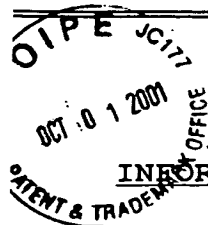
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August 2, 2001

Group Art Unit

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		BA	5,472,687	12/5/95	Proctor		2/7/94
		BB	5,453,437	9/26/95	Schohe et al.		9/8/93
		BC	5,447,915	9/5/95	Schreiber et al.		2/21/91
		BD	5,359,138	10/25/94	Takeuchi et al.		6/29/92
		BE	5,342,942	8/30/94	Jaen et al.		3/29/93
		BF	5,330,993	7/19/94	Armistead et al.		7/2/91
		BG	5,321,009	6/14/94	Baeder et al.		11/14/91
		BH	5,294,603	3/15/94	Rinehart		2/18/92
		BI	5,232,923	8/3/93	Fukazawa et al.		4/22/91
		BJ	5,215,969	6/1/93	Springer et al.		12/9/91
		BK	5,214,034	5/25/93	Nakayama et al.		12/3/91
		BL	5,204,338	4/20/93	Baader et al.		5/10/91
		BM	5,192,773	3/9/93	Armistead et al.		7/2/90
		BN	5,166,317	11/24/92	Wallace et al.		10/31/88
		BO	5,147,877	9/15/92	Goulet		9/12/91
		BP	5,002,962	3/26/91	Loscalzo		6/15/88
		BQ	5,002,963	3/26/91	De Luca et al.		6/1/88
		BR					
		BS					
		BT					

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August 2, 2001Group Art Unit
1614

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CC/cursory review as submitted	CA	4,818,749	4/4/89	Gold et al.			11/4/87
	CB	4,745,124	5/17/88	Ryan et al.			7/20/83
	CC	4,734,420	3/29/88	Ryan et al.			5/2/80
	CD	4,692,458	9/8/87	Ryan et al.			3/5/80
	CE	4,472,380	9/18/84	Harris et al.			9/27/82
	CF						
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	CH						
	CI						
	CJ						
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	CN						Yes No
	CO						Yes No
	CP						Yes No

OTHER (Including Author, Title, Date, Pertinent Pages, etc.)

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		AA	Jou, Chemical Abstracts, vol. 126:89769.
		AB	Burbaum, Chemical Abstracts, vol. 121:109686.
		AC	Rinehart, Chemical Abstracts, vol. 121:887
		AD	Gold, 94:82238 USPATFULL
		AE	Baader, Chemical Abstracts, vol. 121:102790
		AF	Baader, 93:31405 USPATFULL.
		AG	Mashkovskii, Chemical Abstracts, vol. 121:212542.
		AH	Cunliffe, Chemical Abstracts, vol. 117:49183.
		AI	Rinehart, Chemical Abstracts, vol. 115:248086.
		AJ	Baader, Chemical Abstracts, vol. 116:129617.
		AK	Krit, Chemical Abstracts, vol. 115:232847.
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		AM	Gold, Chemical Abstracts, vol. 111:197735.
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		AO	Boulmedais, Chemical Abstracts, vol. 112:44174.
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CC/cursory review as submitted

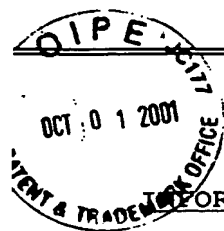
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BG	Neustadt, Chemical Abstracts, vol. 97:216730.
BH	Ryan, Chemical Abstracts, vol. 97:163506.
BI	Patchett, Chemical Abstracts, vol. 95:25634.
BJ	Cushman, Chemical Abstracts, vol. 88:18091.
BK	Steglich, Chemical Abstracts, vol. 85:108966.
BL	Hearn, Chemical Abstracts, vol. 68:22217.
BM	5434118 <u>Beilstein</u> .
BN	5337004 <u>Beilstein</u> .
BO	5059234 <u>Beilstein</u> .

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CA 442355 Beilstein.CB 422738 Beilstein.CC 407472 Beilstein.

CD Armistead, Chemical Abstracts, vol. 126:343875.

CE Armistead, Chemical Abstracts, vol. 127:346300.

CF Holt, Chemical Abstracts, vol. 127:262560.

CG Holt, Chemical Abstracts, vol. 127:247960.

CH Armistead, 97:81322 USPATFULL.

CI Armistead, 97:68499 USPATFULL.

CJ Armistead, 97:33765 USPATEFULL.

CK Amara, Chemical Abstracts, vol. 127:316501.

CL Armistead, Chemical Abstracts, vol. 126:272378.

CM Holt, Chemical Abstracts, vol. 125:86501.

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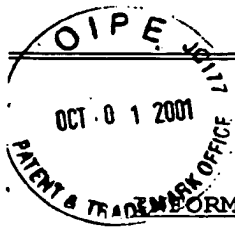
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DB

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DC

Birkenshaw, Chemical Abstracts, vol. 122:187213.

DD

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DE

Yamashita, Chemical Abstracts, vol. 120:315168.

DF

Luengo, Chemical Abstracts, vol. 121:49600.

DG

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DH

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DI

Armistead, Chemical Abstracts, vol. 117:131071.

DJ

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DK

Bender, Chemical Abstracts, vol. 89:128811.

DL

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DM

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DN

6653693 Beilstein.

DO

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EA	Hamilton, 97:25061 USPATFULL.
EB	Hamilton, Chemical Abstracts, vol. 126:144545.
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ED	Steiner, Chemical Abstracts, vol. 126:152817.
EE	Steiner, Chemical Abstracts, vol. 126:152815.
EF	Baldwin, 123:350246 MARPAT.
EG	MacLeod, 121:205217 MARPAT.
EH	Hauske, Chemical Abstracts, vol. 122:45705.
EI	Holt, Chemical Abstracts, vol. 121:224.
EJ	Burakoff, 92:40822 USPATEFULL.
EK	Hauske, Chemical Abstracts, vol. 118:22591.
EL	Schreiber, Chemical Abstracts, vol. 116:34554.
EM	Munegumi, Chemical Abstracts, vol. 113:191883.
EN	Finberg, Chemical Abstracts, vol. 113:184256.
EO	Toda, 113:23894 MARPAT.

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	FC	Roloff, 87:68534 USPATFULL.
	FD	Roloff, Chemical Abstracts, vol. 105:1333746.
	FE	Roloff, 86:35764 USPATFULL.
	FF	Ryan, Chemical Abstracts, vol. 97:163506.
	FG	Haeusler, 1974:569816 ZCAPLUS.
	FH	Pansare, Chemical Abstracts, vol. 123:339285.
	FI	Byun, Chemical Abstracts, vol. 123:111799.
	FJ	Toda, 113:23894 MARPAT.
	FK	Suzuki, Chemical Abstracts, vol. 108:150009.
	FL	Koft, Chemical Abstracts, vol. 106:119239.
	FM	Bycroft, Chemical Abstracts, vol. 84:106021.

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APPENDIX C

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BOX PROVISIONAL PATENT APPLICATION
Attorney Docket No. 23402

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Ella MAGAN

Serial No. not yet assigned

Filed: September 24, 1997

For: METHODS FOR PREVENTING AND TREATING HEARING LOSS USING
NEURO IMMUNOPHILIN LIGANDS

TRANSMITTAL LETTER

The Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Submitted herewith for filing in the U.S. Patent and Trademark
Office is the following:

- (1) Transmittal Letter;
- (2) Check No. 7326 in the amount of \$150.00;
- (3) Provisional Application Filing Form; and
- (4) Provisional Application as filed:
34 pages text (specification, claims, abstract)
6 sheets of figures.

Please charge any required fee, or credit any overpayment, in
connection with this matter to deposit Account No. 14-0112.

Respectfully submitted,

By:

Todd L. Juneau
Todd L. Juneau

Registration No. 40,669

Date: September 24, 1997
NATH & ASSOCIATES
1835 K Street N.W., Suite 750
Washington, D.C. 20006-1203
(202) 775-8383

GHR/TLJ/jc

69564 U.S. PTO
60/059905



PATENT APPLICATION SERIAL NO. 09/24/97

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

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**METHOD FOR PREVENTING AND TREATING HEARING LOSS USING NEUROIMMUNOPHILIN
LIGANDS.**

INVENTOR: ELLA MAGAL

**NATH & ASSOCIATES
1835 K STREET N.W., SUITE 750
WASHINGTON, D.C. 20006
(202) 775-8383**

50059905-109249

METHOD FOR PREVENTING AND TREATING HEARING LOSS USING NEUROIMMUNOPHILIN LIGANDS.

BACKGROUND OF THE INVENTION

The present invention relates generally to methods for preventing and/or treating injury or degeneration of inner ear sensory cells, such as hair cells and auditory neurons, by administering neuroimmunophilins family products. The invention relates specifically to methods for preventing and/or treating hearing loss due to variety of causes.

INTRODUCTION ABOUT NEUROIMMUNOPHILINS

The neuroepithelial hair cells in the organ of Corti of the inner ear, transduce sound into neural activity, which is transmitted along the cochlear division of the eighth cranial nerve. This nerve consists of fibers from three types of neurons (Spoendlin, H. H. In: Friedmann, I. Ballantyne, J., eds. Ultrastructural Atlas of the Inner Ear; London, Butterworth, pp. 133-164, 1984): 1) afferent neurons, which lie in the spiral ganglion and connect the cochlea to the brainstem. 2) efferent olivocochlear neurons, which originate in the superior olivary complex and 3) autonomic adrenergic neurons, which originate in the cervical sympathetic trunk and innervate the cochlea. In the human, there are approximately 30,000 afferent cochlear neurons, with myelinated axons, each consisting of about 50 lamellae, and 4-6 μm in diameter. This histologic structure forms the basis of uniform conduction velocity, which is an important functional feature. Throughout the length of the auditory nerve, there is a

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trophic arrangement of afferent fibers, with 'basal' fibers wrapped over the centrally placed 'apical' fibers in a twisted rope-like fashion. Spoendlin (Spoendlin, H. H. In: Naunton, R. F., Fernadex, C. eds. Evoked Electrical Activity in the Auditory Nervous System. London, Academic Press, pp. 21-39, 1978) identified two types of afferent neurons in the spiral ganglion on the basis of morphologic differences: type I cells (95%) are bipolar and have myelinated cell bodies and axons that project to the inner hair cells. Type II cells (5%) are monopolar with unmyelinated axons and project to the outer hair cells of the organ of Corti. Each inner hair cell is innervated by about 20 fibers, each of which synapses on only one cell. In contrast, each outer hair cell is innervated by approximately six fibers, and each fiber branches to supply approximately 10 cells. Within the cochlea, the fibers divide into: 1) an inner spiral group, which arises primarily ipsilaterally and synapses with the afferent neurons to the inner hair cells, and 2) a more numerous outer radial group, which arises mainly contralaterally and synapses directly with outer hair cells. There is a minimal threshold at one frequency, the characteristic or best frequency, but the threshold rises sharply for frequencies above and below this level (Pickles, J.O. In: Introduction to the Physiology of Hearing. London, Academic Press, pp. 71-106, 1982). Single auditory nerve fibers therefore appear to behave as band-pass filters. The basilar membrane vibrates preferentially to different frequencies, at different distances along its length, and the frequency selectivity of each cochlear nerve fiber is similar to that of the inner hair cell to which the fiber is connected. Thus, each cochlear nerve fiber exhibits a turning curve covering a different range of frequencies from its neighboring fiber (Evans, E. F. In: Beagley H. A. ed. Auditory investigation: The Scientific and Technological basis. New York, Oxford University Press, 1979). By this mechanism, complex sounds are broken down into component frequencies (frequency resolution) by the filters of the inner ear.

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Hearing loss of a degree sufficient to interfere with social and job-related communications is among the most common chronic neural impairments in the US population. On the basis of health-interview data (Vital and health statistics. Series 10. No. 176. Washington, D.C. (DHHS publication no. (PHS) 90-1504), it is estimated that approximately 4 percent of people under 45 years of age and about 29 percent of those 65 years or over have a handicapping loss of hearing. It has been estimated that more than 28 million Americans have hearing impairment and that as many as 2 million of this group are profoundly deaf (A report of the task force on the National Strategic plan. Bethesda, Md.: National Institute of Health, 1989). The prevalence of hearing loss increases dramatically with age. Approximately 1 per 1000 infants has a hearing loss sufficiently severe to prevent the unaided development of spoken language (Gentile, A. et al. Characteristics of persons with impaired hearing: United States, 1962-1963. Series 10. No. 35. Washington, D.C.: Government printing office, 1967 (DHHS publication no. (PHS) 1000) (Human communication and its disorders: an overview. Bethesda, Md.: National Institutes of health, 1970). More than 360 per 1000 persons over the age of 75 have a handicapping hearing loss (Vital and health statistics. Series 10. No. 176. Washington, D.C. (DHHS publication no. (PHS) 90-1504).

It has been estimated that the cost of lost productivity, special education, and medical treatment may exceed \$30 billion per year for disorders of hearing, speech and language (1990 annual report of the National Deafness and other Communication Disorders Advisory Board. Washington, D.C.: Government Printing Office, 1991. (DHHS publication no. (NIH) 91-3189). The major common causes of profound deafness in childhood are genetic disorders and meningitis, constituting approximately 13 percent and 9 percent of the total, respectively (Hotchkiss, D. Demographic aspects of hearing impairment: questions and answers. 2nd ed. Washington, D.C.: Gallaudet University Press, 1989). In approximately 50 percent of the cases of childhood deafness, the cause is unknown, but is likely due to genetic causes or

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predisposition(Nance WE, Sweeney A. Otolaryngol. Clin. North Am 1975; 8: 19-48).

Impairment anywhere along the auditory pathway, from the external auditory canal to the central nervous system, may result in hearing loss. The auditory apparatus can be subdivided into the external and middle ear, inner ear and auditory nerve and central auditory pathways. Auditory information in humans is transduced from a mechanical signal to a neurally conducted electrical impulse by the action of approximately 15,000 neuroepithelial cells (hair cells) and 30,000 first-order neurons (spiral ganglion cells) in the inner ear. All central fibers of spiral ganglion neurons form synapses in the cochlear nucleus of the pontine brainstem. The number of neurons involved in hearing increases dramatically from the cochlea to the auditory brain stem and the auditory cortex. All auditory information is transduced by only 15,000 hair cells, of which the so-called inner hair cells, numbering 3500, are critically important, since they form synapses with approximately 90 percent of the 30,000 primary auditory neurons. Thus, damage to a relatively few cells in the auditory periphery can lead to substantial hearing loss. Hence, most causes of sensorineural loss can be ascribed to lesions in the inner ear (Nadol, J.B., New England Journal of Medicine, 1993, 329: 1092-1102).

Hearing loss can be on the level of conductivity, sensorineural and central level. Conductive hearing loss is caused by lesions involving the external or middle ear, resulting in the destruction of the normal pathway of airborne sound amplified by the tympanic membrane and the ossicles to the inner ear fluids. Sensorineural hearing loss is caused by lesions of the cochlea or the auditory division of the eight cranial nerve. Central hearing loss is due to lesions of the central auditory pathways. These consist of the cochlear and dorsal olivary nucleus complex, inferior colliculi, medial geniculate bodies, auditory cortex in the temporal lobes and interconnecting afferent and efferent fiber tracts (Adams R. D. and Maurice, V.

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Eds. in: Principles of Neurology. 1989. McGraw-Hill Information services Company. PP 226-246).

As mentioned previously, at least 50 percent of cases of profound deafness in childhood have genetic causes (Brown, K. S. Med. Clin. North AM. 1969; 53: 741-72). If one takes into consideration the probability that genetic predisposition is a major causative factor in presbycusis - or age-related hearing loss- which affects one third of the population over 75 years of age (Nadol, J. B. In: Beasley DS, Davis GA, eds. Aging: Communication Processes and Disorders. New York: Grune & Stratton, 1981:63-85), genetic and hereditary factors are probably the single most common cause of hearing loss. Genetic anomalies are much more commonly expressed as sensorineural hearing loss than as conductive hearing loss. Genetically determined sensorineural hearing loss is clearly a major, if not the main cause of sensorineural loss, particularly in children (Nance WE, Sweeney A. Otolaryngol. Clin. North Am 1975; 8: 19-48). Among the most common syndromal forms of sensorineural loss are Waardenburg's syndrome, Alport's syndrome and Usher's syndrome.

A variety of commonly used drugs have ototoxic properties. The best known are the aminoglycoside antibiotics (Lerner, S. A. et al eds. Aminoglycoside ototoxicity. Boston: Little, Brown, 1981; Smith, C. R. et al. N Engl. J. Med. 1980; 302: 1106-9), loop diuretics (Bosher, S. K., Acta Otolaryngol. (Stockh) 1980; 90: 4-54), salicylates (Myers, E. N. at al. N Engl. J. Med. 1965; 273:587-90) and antineoplastic agents such as cisplatin (Strauss, M. at al. Laryngoscope 1983; 143:1263-5). Ototoxicity has also been described during oral or parenteral administration of erythromycin (Kroboth, P. D. at al. Arch. Intern Med. 1983; 113:169-79; Achweitzer, V. G., Olson, N. Arch. Otolaryngol. 1984; 110:258-60).

Most ototoxic substances cause hearing loss by damaging the cochlea, particularly the auditory hair cells and the stria vascularis, a specialized epithelial organ within the inner ear, that is responsible for the homeostasis of fluids and

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electrolytes (Nadol, J.B. New England J. Med. 1993; 329: 1092-1102). Secondary neural degeneration may occur many years after an ototoxic event affecting the hair cells. There is evidence that some ototoxic substances may be selectively concentrated within the inner ear, resulting in progressive sensorineural loss despite the discontinuation of systemic administration (Federspil, P. et al. J. Infect. Dis. 1976; 134 Suppl: S200-S205).

Trauma due to acoustic overstimulation is another leading cause of deafness. There is individual susceptibility to trauma from noise. Clinically important sensorineural hearing loss may occur in some people exposed to high-intensity noise, even below levels approved by the Occupational Safety and Health Agency (Osguthorpe, J. D. ed. Washington D.C.: American Academy of Otolaryngology-Head and Neck Surgery Foundation, 1988).

Demyelinating processes, such as multiple sclerosis, may cause sensorineural hearing loss (Noffsinger, D et al. Acta Otolaryngol Suppl (Stockh) 1972; 303:1-63). More recently, a form of immune-mediated sensorineural hearing loss has been recognized (McCabe, B. F. Ann Otol Rhinol Laryngol 1979; 88:585-9). The hearing loss is usually bilateral, is rapidly progressive (measured in weeks and months), and may or may not be associated with vestibular symptoms.

A variety of tumors, both primary and metastatic, can produce either a conductive hearing loss, or a sensorineural hearing loss, by invading the inner ear or auditory nerve (Houck, J. R. et al. Otolaryngol Head Neck Surg 1992; 106:92-7). A variety of degenerative disorders of unknown cause can produce sensorineural hearing loss. Meniere's syndrome (Nadol, J. B. ed. Meniere's disease: pathogenesis, pathophysiology, diagnosis, and treatment. Amsterdam: Kugler & Ghedini 1989), characterized by fluctuating sensorineural hearing loss, episodic vertigo, and tinnitus, appears to be caused by a disorder of fluid homeostasis within the inner ear, although the pathogenesis remains unknown. Sudden idiopathic sensorineural hearing loss (Wilson, W. R. et al. Arch Otolaryngol 1980; 106:772-6), causing moderate-to-severe

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sensorineural deafness, may be due to various causes, including inner ear ischemia and viral labyrinthitis.

Presbycusis, the hearing loss associated with aging, affects more than one third of persons over the age of 75 years. The most common histopathological correlate of presbycusis is the loss of neuroepithelial (hair) cells, neurons, and the stria vascularis of the peripheral auditory system (Schuknecht H. F. Pathology of the Ear. Cambridge, Mass: Harvard University Press, 1974:388-403). Presbycusis is best understood as resulting from the cumulative effects of several noxious influences during life, including noise trauma, ototoxicity and genetically influenced degeneration.

VESTIBULAR DISORDERS - add introduction

TINNITUS - add introduction

SUMMARY OF THE INVENTION

The present invention provides methods for treating sensorineural hearing loss comprising administering to a subject having a lesion in the inner ear a therapeutically effective amount of a neuroimmunophilins family small molecules. For example, the hearing loss may be associated with injury or degeneration of neuroepithelial hair cells (cochlear hair cells) or spiral ganglion neurons in the inner ear.

The present invention is based on the discoveries that hair cells respond to neuroimmunophilins family small molecules (GPI1046) by resisting the toxic effects of ototoxins, such as cisplatin and neomycin. Thus, a therapeutically effective amount neuroimmunophilins family small molecules may be administered to promote the protection, survival or regeneration of hair cells and spiral ganglion neurons.

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It has also been discovered that lesions or disturbances to the vestibular apparatus which sensory transducing organ consists of the same hair cells as the cochlea, may also be treated by administering to a subject having such a lesion or disturbance a therapeutically effective amount of a neuroimmunophilins family small molecules. Such lesions may result in dizziness, vertigo or loss of balance.

According to the invention, the neuroimmunophilins family small molecules may be administered parenterally at a dose ranging from about 1 ng/ear/day to about 10 ng/ear/day, typically at a dose of about 1 µg/ear/day to about 10 µg/ear/day, and usually at a dose of about 5 mg/kg/day to about 20 mg/kg/day. It is also contemplated that, depending on the individual patient's needs and route of administration, the neuroimmunophilins family small molecules may be given at a lower frequency such as monthly, weekly or several times per week, rather than daily. It is further contemplated that neuroimmunophilins family small molecules may be administered directly into the middle ear or the inner ear. One skilled in the art will appreciate that with such administration of a smaller amount of neuroimmunophilins family small molecules may be used, for example, a direct middle ear or inner-ear administration dose in the range of about 1 ng/ear to about 10 ng/ear in a single injection or in multiple injections.

It is further contemplated that neuroimmunophilins family small molecules be administered in combination or conjunction with an effective amount of a second therapeutic agents, such as GDNF, BDNF and NT-3. The invention also provides for the use of neuroimmunophilins family small molecules in the manufacture of a medicament or pharmaceutical composition for the treatment of injury or degeneration of hair cells and auditory neurons for the variety of causes of sensorineural hearing loss. Such pharmaceutical compositions include topical, oral or middle and inner ear neuroimmunophilins family small molecules formulations or in combination with cochlear implants.

2025 RELEASE UNDER E.O. 14176

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for preventing and/or treating sensorineural hearing loss by administering a therapeutically effective amount of neuroimmunophilins family small molecules. According to one aspect of the invention, methods are provided for treating damaged hair cells and auditory neurons by administering a therapeutically effective amount of neuroimmunophilins family small molecules by means of a pharmaceutical composition. The invention may be practiced using any biologically active neuroimmunophilins family small molecules.

The present invention is based on the initial discoveries that neuroimmunophilins family small molecules protect hair cells from ototoxins-induced cell death in explant cultures of rat's cochlea and in animal model (guinea pig) of deafness. It is contemplated that administration of exogenous neuroimmunophilins family small molecules will protect hair cells and spiral ganglion neurons from traumatic damage (such as noise trauma and acute or chronic treatments of cisplatin and aminoglycoside antibiotics) or from damage resulting from a lack of neurotrophic factors caused by interruption of transport of the factors from the axon to the cell body. Such treatment is expected to allow hair cells and /or auditory neurons to tolerate intermittent insults from either environmental noise trauma, treatments with ototoxins and to slow down, prevent or reverse the progressive degeneration of the auditory neurons and hair cells, that is responsible for hearing loss in pathological conditions such as presbycusis (age-related hearing loss), inherited sensorineural degeneration, and post-idopathic hearing losses and to preserve the functional integrity of the inner ear. It will also support

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the auditory neurons for a better and longer performance of cochlear implants.

According to the invention, the neuroimmunophilins family small molecules may be administered into the middle ear at a dose ranging from about 1 ng/ear/day to about 10 ng/ear/day, typically at a dose of about 1 μ g ear/day to about 10 μ g/ear/day, and usually at a dose of about 5 μ g/ear/day to about 20 μ g/ear/day. GDNF protein neuroimmunophilins family small molecules may be administered directly into the inner ear in cases where invasion of the inner ear is already in place such as in the procedure of cochlear implant or surgeries of the inner ear. In such cases, a smaller amount of neuroimmunophilins family small molecules will be administered, for example, from about 0.1 ng/ear to about 1 ng/ear in a single injection or in multiple injections. Neuroimmunophilins family small molecules can be also developed in a form of ear-drops which will penetrate the tympanic membrane of the Bulla. It is further contemplated that neuroimmunophilins family small molecules be administered with an effective amount of a second therapeutic agent for the treatment of auditory neuron degeneration, together with GDNF, BDNF and NT-3 as well as other factors or drugs used currently or in the future for the treatment of the various inner ear pathologies. A variety of pharmaceutical formulations and different delivery techniques are described in further detail below.

As used herein, the term " neuroimmunophilins family small molecules " includes all biologically active synthetic compounds that bind to the protein receptor FKBP12 and chemically modified derivatives thereof.

The term "biologically active" as used herein means that the neuroimmunophilins family small molecules demonstrates similar neurotrophic properties, but not necessarily all of the same properties, and not necessarily to the same degree, as the GPI1046.

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Neuroimmunophilins family small molecules may also be chemically synthesized by any means known to those skilled in the art.

C. Neuroimmunophilins family small molecules Pharmaceutical Compositions

Neuroimmunophilins family small molecules pharmaceutical compositions typically include a therapeutically effective amount of neuroimmunophilins family small molecules in admixture with one or more pharmaceutically and physiologically acceptable formulation materials. Suitable formulation materials include, but are not limited to, antioxidants, preservatives, coloring, flavoring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. For example, a suitable vehicle may be water for injection, physiological saline solution, or artificial perilymph, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles.

The primary solvent in a vehicle may be either aqueous or non-aqueous in nature. In addition, the vehicle may contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the vehicle may contain still other pharmaceutically-acceptable excipients for modifying or maintaining the rate of release of GDNF protein product, or for promoting the absorption or penetration of GDNF protein product across the tympanic membrane. Such excipients are those substances usually and customarily employed to formulate dosages for middle-ear administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel,

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emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or in a form, e.g., lyophilized, requiring reconstitution prior to administration.

The optimal pharmaceutical formulations will be determined by one skilled in the art depending upon considerations such as the route of administration and desired dosage. See for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712, the disclosure of which is hereby incorporated by reference. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the present GDNF proteins, variants and derivatives.

Other effective administration forms, such as middle-ear slow-release formulations, inhalant mists, or orally active formulations are also envisioned. For example, in a sustained release formulation, the neuroimmunophilins family small molecules may be bound to or incorporated into particulate preparations of polymeric compounds (such as polylactic acid, polyglycolic acid, etc.) or liposomes. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. The neuroimmunophilins family small molecules pharmaceutical composition also may be formulated for middle-ear administration, e.g., by tympanic membrane infusion or injection, and may also include slow-release or sustained circulation formulations. Such middle-ear administered therapeutic compositions are typically in the form of a pyrogen-free, middle-ear acceptable aqueous solution comprising the neuroimmunophilins family small molecules in a pharmaceutically acceptable vehicle. One preferred vehicle is sterile distilled water.

It is also contemplated that certain formulations containing neuroimmunophilins family small molecules are to be administered orally. neuroimmunophilins family small molecules which is administered in this fashion may be encapsulated and may be formulated with or without those carriers customarily used in the

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compounding of solid dosage forms. The capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional excipients may be included to facilitate absorption of neuroimmunophilins family small molecules. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

The formulation of topical ear preparations, including middle-ear solutions, suspensions and ointments is well known to those skilled in the art (see Remington's Pharmaceutical Sciences, 18th Edition, Chapter 86, pages 1581-1592, Mack Publishing Company, 1990). Other modes of administration are available, including injections to the middle ear, and methods and means for producing middle-ear preparations suitable for such modes of administration are also well known.

As used in this application, "middle-ear" refers to the space between the tympanic membrane and the inner ear. This location is external to all inner ear tissue and an invasive procedure might not be required to access this region if a formulation will be developed so that the neuroimmunophilins family small molecules will penetrate through the tympanic membrane. Otherwise, the material will be introduced to the middle ear by injection through the tympanic membrane or, in case repeated administrations are needed, a hole will be made in the tympanic membrane. Examples of such systems include inserts and "topically" applied drops, gels or ointments which may be used to deliver therapeutic material to these regions. An opening in the tympanic membrane is a very frequent procedure done on a office-visit basis, in cases such as infections of the middle ear (usually in children). The opening closes spontaneously after a few days.

In the presently described use of neuroimmunophilins family small molecules of the treatment of inner ear disease or injury it is also advantageous that a topically applied formulation include an agent to promote the penetration or transport of the

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therapeutic agent into the middle and inner ear. Such agents are known in the art. For example, Ke et al., U.S. 5,221,696 disclose the use of materials to enhance the penetration of ophthalmic preparations through the cornea.

Inner-ear systems are those systems which are suitable for use in any tissue compartment within, between or around the tissue layers of the inner-ear, such as the cochlea and vestibular organ. These locations include the different structures of the cochlea such as the stria vascularis, Reissner's membrane, organ of Corti, spiral ligament and the cochlear neurons. An invasive procedure might not be required to access those structures since it has been shown that even proteins, let alone small molecules, do penetrate the membrane of the round window into the perilymph of the inner ear. A particularly suitable vehicle for introducing neuroimmunophilins family small molecules into the inner ear by penetration through the round window membrane is artificial perilymph. This solution consists of 10.00 mM D-glucose, 1.5 mM CaCl₂, 1.5 mM MgCl₂ in a 1.0% solution of Dulbecco's phosphate-buffered saline in deionized water at 280-300 mOsm and pH of 7.2. Yet another preparation may involve the formulation of the neuroimmunophilins family small molecules with an agent, such as injectable microspheres or liposomes into the middle ear, that provides for the slow or sustained release of the molecules which may then be delivered as a depot injection. Other suitable means for the inner-ear introduction of neuroimmunophilins family small molecules includes, implantable drug delivery devices or which contain the neuroimmunophilins family small molecules, and a cochlear-implant with a tunnel through, so neuroimmunophilins family small molecules can be continuously delivered through it to the inner ear.

The ear-treatment preparations of the present invention, particularly topical preparations, may include other components, for example middle-ear acceptable preservatives, tonicity agents, cosolvents, complexing agents, buffering agents, antimicrobials, antioxidants and surfactants, as are well known in the art. For

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example, suitable tonicity enhancing agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol and the like. Sufficient tonicity enhancing agent is advantageously added so that the formulation to be instilled into the ear is compatible with the osmolarity of the endo- and perilymph. Suitable preservatives include, but are not limited to, benzalkonium chloride, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and the like. Hydrogen peroxide may also be used as preservative. Suitable cosolvents include, but are not limited to, glycerin, propylene glycol and polyethylene glycol. Suitable complexing agents include caffeine, polyvinylpyrrolidone, beta -cyclodextrin or hydroxypropyl- beta -cyclodextrin. The buffers can be conventional buffers such as borate, citrate, phosphate, bicarbonate, or Tris-HCl.

The formulation components are present in concentration that are acceptable to the middle or inner ear site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

Additional formulation components may include materials which provide for the prolonged the residence of the middle ear administered therapeutic agent so as to maximize the topical contact and promote absorption through the round window membrane. Suitable materials include polymers or gel forming materials which provide for increased viscosity of the middle-ear preparation. The suitability of the formulations of the instant invention for controlled release (e.g., sustained and prolonged delivery) of an inner-ear treating agent can be determined by various procedures known in the art. Yet another ear preparation may involve an effective quantity of neuroimmunophilins family small molecules in a mixture with non-toxic middle-ear treatment acceptable excipients which are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, middle-ear treatment solutions can be prepared in unit dose form. Suitable excipients include, but are

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not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia.

Administration/Delivery of neuroimmunophilins family small molecules

The neuroimmunophilins family small molecules may be administered parenterally via a subcutaneous, intramuscular, intravenous, transpulmonary, transdermal, intrathecal or intracerebral route. For the treatment of inner-ear conditions, the neuroimmunophilins family small molecules may be administered into the middle-ear (or directly into the inner-ear, especially in cases where invasive procedure is already in place), by topical application, inserts, injection and implants. For example, slow-releasing implants containing the molecules embedded in a biodegradable polymer matrix can deliver neuroimmunophilins family small molecules.. Neuroimmunophilins family small molecules may be administered in the middle or inner ear, or it may be administered on top of the tympanic membrane in connection with one or more agents capable of promoting penetration or transport of neuroimmunophilins family small molecules across the membranes of the ear. The frequency of dosing will depend on the pharmacokinetic parameters of the neuroimmunophilins family small molecules as formulated, and the route of administration.

The specific dose may be calculated according to considerations of body weight, body surface area or organ size. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed, especially in light of the dosage information and assays disclosed herein. Appropriate dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data. It will be appreciated by those skilled in the art that the dosage

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used in inner-ear administered formulations will be minuscule as compared to that used in a systemic injection or oral administration.

The final dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g., the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding the appropriate dosage levels for the treatment of various diseases and conditions.

It is envisioned that the continuous administration or sustained delivery of neuroimmunophilins family small molecules may be advantageous for a given treatment. While continuous administration may be accomplished via a mechanical means, such as with an infusion pump, it is contemplated that other modes of continuous or near continuous administration may be practiced. For example, subcutaneous and muscular injections as well as oral pills and ear drops.

Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible particles or beads and depot injections, are also known to those skilled in the art.

It should be noted that the neuroimmunophilins family small molecules formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges should be the same as specified above.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples. Example 1 and 2 addresses the effects of neuroimmunophilins family small molecules (GPI1046) administration on hair cells in a Cochlear explant culture

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system. Example 3 and 4 addresses the effects of neuroimmunophilins family small molecules administration on hair cells in cochlea of guinea pigs treated with various clinically relevant ototoxins such as neomycin and cisplatin. The results of the organ of Corti explant cultures studies and that of the animal model of deafness demonstrated that neuroimmunophilins family small molecules has protective activity for the hair cells of the organ of Corti against ototoxins, which were not previously known to be neuroimmunophilins family small molecules responsive.

EXAMPLES

EXAMPLE 1

GPI1046 Protects Cochlear Hair Cells Against Ototoxicity induced by neomycin and cisplatin - In Vitro

MATERIALS

The materials used in the following Example were obtained as follows.

Organ of Corti dissecting solution:

Dulbecco's Phosphate Buffered Saline (PBS; 1x, without calcium chloride, without magnesium chloride. Cat. #14190-136, Gibco BRL), containing 1.5 g/L D-Glucose (Dextrose. Cat. #15023-021, Gibco BRL).

Organ of Corti explant culture Medium

1. High glucose Dulbecco's Modified Eagle Medium (DMEM; 1 X , with L-glutamine, without Sodium Pyruvate. Cat. #11965-084, Gibco BRL)
2. 0.15 g/100 ml of D-Glucose (Dextrose. Cat. #15023-021, Gibco BRL)

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3. 1% N-2 Supplement (100 X, Cat. #17502-030, Gibco BRL)
4. 100 Units/ml of Penicillin G, Potassium (Penicillin; Cat. # 21840-020, Gibco BRL)

METHODS

Preparation of Medium

DMEM was supplemented with 1 % N-2 supplement, and D-glucose was added to a final concentration of 1.5 g/L. Penicillin was added at 100 Units/ml. After mixing, the medium was filtered and kept at 4°C. The medium was prepared fresh just before use in order to minimize inter-experimental variations. Plastic pipettes and containers were used throughout to minimize protein adsorption.

Dissecting tools and culture dishes

1. The 4" and 5" dissecting forceps and 4" dissecting scissors were from Roboz Surgical, Washington, DC.
2. Falcon sterile 96-well microplates (Flat Bottom. Cat. #3072), tissue culture plastic ware and polypropylene centrifuge tubes were from Beckton-Dickinson, Lincoln Park, New Jersey.

GPI1046 Product Solutions

GPI1046 stock was stored in room temperature and prepared fresh for each culture. GPI1046 stock solution was diluted in 10 μ l of 100% EtOH for every milligram of GPI1046 stock (approximately 250mM). This solution of 250mM GPI 046 in 100% EtOH was diluted in normal culture medium to working concentrations of 50000 nM, 5000nM, 500nM, 50nM, 5000pM, 500pM, 50pM, 10pM, 5pM, 1pM, 0.5pM, 0.1pM, and 0.01pM. Ten microliters of ten-fold concentrated GPI 1046 product solutions were added to Organ of Corti explant cultures containing ototoxin

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medium (90 μ l), so that the final GPI 1046 concentration were 5000nM, 500nM, 50nM, 5nM, 500pM, 50pM, 5pM, 1pM, 0.5pM, 0.1pM, 0.05pM, 0.01pM, and 0.001pM. Control cultures received normal medium (10 μ l). The GPI1046 product treatments were initiated at first day culture (one day before ototoxin treatment), and repeated with ototoxin treatment at second day.

Ototoxins and Related Reagents

1. Neomycin solution (Cat. #N1142, Sigma. St. Louis, MO), used at final concentration of 0.6 mM (A fresh solution was made each experiment by adding 90 μ l of 1mg/ml neomycin and to 1410 μ l medium).
2. Cisplatin (Platinol-AQ. Cat. #NDC 0015-3220-22, Bristol-Myers Squibb Laboratories, Princeton, New Jersey). Used at a final concentration of 35 μ g/ml (a fresh solution was prepared each experiment by adding 52.5 μ l of 1 mg/ml cisplatin to 1447.5 μ l medium)
3. Triton X-100 (t-Octylphenoxypoly-ethoxyethanol. Cat. #X-100, Sigma. St. Louis, MO)
4. Phalloidin (FITC Labeled. Cat. #P-5282, Sigma. St. Louis, MO)
5. Vectashield (Mounting Medium, Cat. #H-1000, Vector, Burlingame, CA)

Preparation of Rat Organ of Corti explant

Organ of Corti explants were obtained from P3-P4 Wistar rats. Rats were decapitated, the lower jaw was cut out and skin removed. The temporal bone was collected in dissection solution, the otic capsule exposed and the bony-cartilaginous cochlear capsule was carefully separated from the temporal bone. Freed cochlea were transferred to another Petri dish with dissection solution for further dissection. Intact organs of Corti were obtained by using a fine forceps to hold central VIII nerve tissue and remove it out,

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then the stria vascular membrane was carefully stripped off, starting from the apex or base. The organ of Corti then was then transferred to a 35-mm diameter Petri dish containing cold PBS supplemented with glucose and ready to be cultured.

Cochlea explant culture procedure

Cochlea explants were cultured in uncoated 96 microplates. A single organ of Corti was placed in a well and was kept floating in the medium. Explants were kept in normal medium for 24 hours (90 μ l/well). GPI1046 solution (10 μ l) was added to the 'treated' cultures and 10 μ l medium was added to controls. After 24 hours of incubation, the media were changed and the explants were exposed to ototoxin-containing medium (90 μ l), with GPI1046 solution (10 μ l) or without (control). The cultures were incubated for an additional 3 days. The explants were then fixed with 4 % paraformaldehyde in 0.1 M D-PBS for 30 minutes at room temperature and processed for immunostaining.

FITC-phalloidin staining of hair cells

To identify and count hair cells in the organ of Corti, a direct immunostaining method was used to label the actin present naturally in the stereocilia bundles of the hair cells. The explants were washed three times with D-PBS (200 μ l per well) and permeabilized with 1 % Triton X-100 in D-PBS for 15 minutes at room temperature. After three washes in D-PBS, the explants were incubated with FITC-labeled Phalloidin (1:60 from stock, 50 μ l/well) for 45 minutes at room temperature. The plates were covered with aluminum foil as the Phalloidin is light sensitive. After three more washes with D-PBS, the labeled explants were placed in a drop of glycerol on a microscope slide, covered with a glass coverslip and sealed with nail polish. The explants were observed under a Nikon Diaphot-300 inverted

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fluorescence microscope, using FITC filters and fluorescence optics.

Determination of hair cell number

For each experimental point, 2 to 4 cochlea were used. In each cochlea, the number of hair cells was counted in 2-3 section, 175 μm in length each. Only the sections in the middle turn of the cochlea were analyzed. Each experiment was repeated several times. The numbers of hair cells in control and cisplatin- or neomycin-treated cultures was generated from analyzing 40 cochlea per point.

RESULTS

Hair cells in the floating explant cultures did not die during the experiment period of four days. Thus, the number of phalloidin-stained cells present at the end of the 4 days experiment period, in the absence of ototoxins and treatments, was 105.4 ± 6.9 ($n=28$). Ototoxins added to the explants on the second day post-plating caused a very significant loss in hair cell number found after 4 days *in vitro*. Exposure to 35 $\mu\text{g/ml}$ cisplatin 24 hours after plating caused a loss of more than 80 percent of the hair cells: only $17.6 \% \pm 5.1$ ($n=20$) of the initial number of hair cells survived (Figure 1) and after exposure to 0.6 mM neomycin, only $5.0 \% \pm 3.8$ ($n=26$) of the hair cells survived (Figure 2).

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There was a marked difference in the morphology of the organs of Corti between this two treatments: while the treatment with neomycin resulted in almost complete loss of hair cells, those that were spared were still organized in the typical four row structure (3 rows of outer hair cells and one row of inner hair cells). Cisplatin treatment, on the other hand, caused a marked disruption of the four-row-structure and the surviving cells were randomly located, indicating a damage caused also to the supporting cells underlying the hair cells.

In cultures that received GPI1046 at the time of plating (pretreatment), a significant number of hair cells survived the 3-day exposure to ototoxins (from day 2 to day 4). In cultures exposed to cisplatin, treatment with GPI1046 at concentrations as low as 0.05 pM resulted in an increase in surviving hair cells from the 17% of the untreated to 41.4%. This, however, was already the maximal activity of GPI1046 as the effect did not titrate out along the range of concentration tested (0.05 pM - 50 nM). Cultures that received neomycin showed reduction of 95% in hair cells compared to controls. Treatment with GPI1046 together with the neomycin reduced this loss to around 70% ($31.8\% \pm 16.4$ surviving hair cells) at a concentration of 0.05 pM, an effect which again did not titrate out nor was increased with higher concentrations of GPI1046.

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EXAMPLE 2

Intramiddle Ear Administered GPI1046 Protects Hair
Cells Against intramiddle Ear Neomycin-induced
Ototoxicity

MATERIALS

The materials used in the following Example were obtained as follows:

Ototoxins - Neomycin sulfate: (Cat. #N-1876, Sigma, St. Louis, MO)

immunophilin ligand - GPI1046: supplied by Guilford.

Vehicle - 20% Intralipid: Intralipid is a 20% I.V. fat emulsion (Cat. #NDC 0338-0491-02, Pharmacia Inc., Clayton, NC). Each 100 ml contains: Soybean oil 20.0 g, Phospholipids (from powdered egg yolk) 1.2 g, Glycerin, USP 2.25 g, Water for injection qs, and Calories 200 kcal. pH 8.0 (6.0-8.9), adjusted with sodium hydroxide.

Ethyl alcohol: 200 proof Dehydrated alcohol, USP (Quantum Chemical Company, Tuscola, IL)

Saline solution: 0.9% sterile sodium chloride aqueous solution (Cat #NDC 57319-077-06, Phoenix Pharmaceutical, Inc., St. Joseph, Missouri)

Gelfoam: absorbable gelatin sponge, USP (Cat. #NDC 0009-0396-01, Upjohn, Kalamazoo, MI)

Guinea pigs: Female pigmented guinea pigs (more sensitive than albino to the ototoxicity induced by aminoglycoside antibiotics) from NIH, body weight: 300-400 g

Phalloidin: FITC Labeled. (Cat. #P-5282, Sigma. St. Louis, MO)

Vectashield: Mounting Medium. (Cat. #H-1000, Vector, Burlingame, CA)

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METHODS

1. The First Middle Ear Administration of GPI1046:

20 Guinea pigs used in this study were divided into two groups: 10 animals received 10 ng and 10 received 1 ng of GPI1046.

Preparation of GPI1046: On the day of compound use, GPI1046 stock solutions were prepared as following:

GPI1046 stock a: a stock solution of GPI1046 at 1 mg/10 ml in 100% ethanol was firstly prepared and then it was diluted and mixed in Intralipid at 10 ng/100 μ l.

GPI1046 stock b: a stock solution of GPI1046 at 1 mg/100 ml in 100% ethanol was firstly prepared and then it was diluted and mixed in 20% Intralipid at 1 ng/100 μ l.

Above stock solutions were made fresh daily, and discard after use.

The vehicle was 20% Intralipid.

The Middle Ear Administration:

Animals were anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and xylazine (4 mg/kg). Through a post-auricular incision, the right bulla was identified. A hole was drilled to open the middle ear cavity (care was taken not to injure the tympanic annulus or ossicles). A piece of gelfoam (~2mm³) was soaked with GPI1046 solution and was inserted into the round window niche. The remain GPI1046 solution (~100 μ l) was then injected into the middle ear cavity. In the 10 ng dose group, 100 μ l of GPI1046 stock a was administered; and in the 1 ng dose group, 100 μ l of GPI1046 stock b was administered to the middle ear cavity. The hole was covered with a piece of clear plastic sheet which was stuck on the skull with a superglue. The incision was closed with clippers. The same procedure was performed at the left bulla, but

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administered with 100 μ l of vehicle solution instead of GPI1046. The animals were maintained in the prone position until they woke up to verify filling of the middle ear cavity.

2. The Second Middle Ear Administration of GPI1046 and Neomycin Ototoxin:

After two days, the animals received the second administration of GPI1046 or vehicle together with neomycin in the middle ears. Solutions were prepared for above two groups of animals as following:

Solution a: Neomycin was dissolved in GPI1046 stock a described above. The final concentration of neomycin was 5 mg and the GPI1046 was 10 ng in a 100 μ l vehicle solution.

Solution b: Neomycin was dissolved in GPI1046 stock b described above. The final concentration of neomycin was 5 mg and the GPI1046 was 1 ng in a 100 μ l vehicle solution.

Solution c: Neomycin was dissolved in 20% Intralipids to final concentration of 5 mg in 100 μ l vehicle.

When the incision was reopened, the plastic cover sheet on the bulla window was pilled off. The old GPI1046 or vehicle were sucked off and the old gelfoam was removed from the round window niche. A piece of gelfoam with fresh stock solution containing GPI1046 and neomycin was administered to the round window niche, and the remaining solution (~100 μ l, solution a for the 10 ng dose group and solution b for the 1 ng dose group respectively) was injected to the middle ear cavity of the right bulla.

solution c (100 μ l) was administered to the left ear for both groups in the same way.

The animals were maintained in the prone position until waking up - to verify filling of the middle ear cavity.

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3. Perfusion And Fixation:

14 days after the second surgery, animals were perfused transcardially with a PBS flush following by a fixative of 4% paraformaldehyde in 0.1M PBS. Immediately following the perfusion, the temporal bone was removed from the head. The bulla was opened and the cochlea was exposed. The apex was opened and the membrane of the round and oval windows was punched. The fixative solution was gently infused into the perilymphatic space through the apex hole and then allowed to flow out from windows. Then the cochleae were post-fixed in the same fixative solution for at least one day.

4. FITC-Phalloidin Staining of Hair Cells:

To identify and count hair cells in the organ of Corti, a direct immunostaining method was used to label the actin present naturally in the stereocilia bundles of the hair cells. The cochlea was dissected and the perilympanic space was fully exposed. The samples were washed three times with PBS (1 ml per well) and permeabilized with 1% Triton X-100 in PBS for 10 minutes at room temperature. After three washes in PBS, The cochlea samples were incubated with FITC-labeled Phalloidin (1:60 from stock, i.e. 1.67 μ g/ml in concentration, 1 ml/well) for 45 minutes at room temperature. The plates were covered with aluminum foil as the Phalloidin is light sensitive. After three more washes with PBS, the labeled cochleas were then bisected and all four turns were removed by microdissection, preserving the hook portion of the basal turn. The turns were mounted on a coverslip (24x60 mm) with Vectashield mounting medium, covered with a glass coverslip and sealed with nail polish. The cochlea turns were observed under a Nikon Diaphot-300 inverted fluorescence microscope, using FITC filters

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and fluorescence optics. The missed outer hair cells (OHC) were counted in every 175 μ m segments (containing 20 OHCs in each row of OHC) beginning at the apex and continuing toward the base.

5. Determination of Hair Cell Number

The cochlea turns were observed under a Nikon Diaphot-300 inverted fluorescence microscope, using FITC filters and fluorescence optics. In each cochlea, the number of missed outer hair cells (OHC) was counted in each 175 μ m segments (containing 20 OHCs in each row of OHC) beginning from the apex and continuing toward the base. The numbers were filled in a cochleogram form for analysis the percentage of OHC loss in each rows, each turns and in whole cochlea of left and right ears. There are four turns per cochlea, the apex called turn 1 is counted top 3.5 mm in length, middle turns including turns 2 (counted 3.5mm-7.0 mm from apex) and turn 3 (7.0mm-10.5mm from apex), and the basal turn called turn 4 (10.5mm-14.0mm).

RESULTS

Table 1 and Figure 1-A show that there was a large and very significant difference in the number of OHCs loss between vehicle and GPI1046 treatments after exposure to ototoxins. In animals that received pretreatment of either 10 ng or 1 ng GPI1046, a significant ($p < 0.0001$, t-test) number of hair cells survived after exposure to ototoxins. Maximal protective activity was on the basal turns (Figure 1-B & C). The results indicate that under this experimental paradigm GPI1046 is capable to completely protect hair cells against ototoxicity.

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Table 1 GPI1046 Protects OHC Loss (%) in Intramiddle Ear Administered Neomycin-induced Hearing Loss Models

treatment time	Left - vehicle mean±SEM	Right- GPI 1046 (mean±SEM	t-test
10 ng GPI1046	86.78±6.81	11.44±7.27	p<0.0001
n=9	73.36± 1.12	15.47± 6.05	p<0.0001
turn-1	94.72± 5.59	13.93±10.75	p<0.0001
turn-2	90.10±10.50	11.64±10.85	p<0.0001
turn-3	88.94±11.73	4.69± 2.85	p<0.0001
turn-4			
1 ng GPI1046	72.14±11.19	3.86±0.37	p<0.0001
n=7	56.11± 9.90	8.85±1.47	p<0.0001
turn-1	72.96±13.97	4.01±0.53	p<0.0001
turn-2	74.07±11.59	0.92±0.10	p<0.0001
turn-3	85.43± 4.82	1.67±1.25	p<0.0001
turn-4			

The results indicate that intramiddle ear administered neomycin caused a marked disruption of the four-row-structure and the surviving cells were randomly located. While the treatment with neomycin and vehicle resulted in almost complete loss of hair cells in most animals, four of 19 animals only lost between ~10% to 37% of OHCs in the same manner (Figure 4-A, B).

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Example 3

**Systemic Administered GPI-1046 Protect Hair Cells
Against Ototoxicity
Induced by Intramiddle Ear Administered Neomycin**

1. MATERIALS

The materials used in the following Example were same as described in the sample 1.

2. METHODS**A. Systemic Administration of GPI-1046:**

20 guinea pigs were treated either with GPI-1046 or vehicle prior to acception of ototoxin. Ten of them were subcutaneously injected with fresh made GPI-1046 solution. On the day of injection, 100 mg of GPI-1046 was dissolved in 1 ml of ethanol, then added 20% of the Intralipids solution into it to the final volume at 3 ml. The final GPI-1046 concentration was 10 mg/0.3 ml. Each animal was subcutaneously injected with 0.3 ml of GPI-1046 solution at day 0, day 2 and day 7 of the experimental schedule. Another 10 animals were subcutaneously injected with 0.3 ml of vehicle, 20% Intralipids, individually at day 0, day 2 and day 7 of the experimental schedule too.

B. Middle Ear Administration of Neomycin:

At the day 2, guinea pigs used in this study were administered neomycin or vehicle in middle ear.

Animals were anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and

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xylozine (4 mg/kg). Through a post-auricular incision, the right bulla was identified. A hole was drilled to open the middle ear cavity (care was taken not to injure the tympanic annulus or ossicles). A piece of gelfoam ($\sim 2\text{mm}^3$) was soaked with neomycin solution (fresh made at the concentration of 50 mg/ml) and was inserted into the round window niche. The remain neomycin solution ($\sim 100\ \mu\text{l}$) was then injected into the middle ear cavity. So total 5 mg of neomycin was applied to the right middle ear. The hole was covered with a piece of clear plastic sheet which was stuck on the skull with a superglue. The incision was closed with clippers. The same procedure was performed at the left ear, but administered with vehicle solution (100 μl of 0.9% saline) instead of neomycin. The animals were maintained in the prone until they woke up to verify filling of the cavity.

C. Perfusion And Fixation:

At the 16th day, animals were perfused transcardially with a PBS flush following by a fixative of 4% paraformaldehyde in 0.1M PBS. Immediately following the perfusion, the temporal bone was removed from the head. The bulla was opened and the cochlea was exposed. The apex was opened and the membrane of the round and oval windows was broken. The fixative solution was infused into the perilymphatic space of the cochlea, and the fixative solution was gently irrigated through the apex hole and then allowed to flow out from windows. Then the cochleae were post-fixed in the same fixative solution for at least one day.

D. FITC-Phalloidin Staining of Hair Cells:

Same in example 2

E. Determination of Hair Cell Number

Same as example 2

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3. RESULTS

A. Protection Effects of Systemic administered GPI-1046 against Neomycin-induced Hair Cell Loss:

Right ears (neomycin treated) from both groups - the vehicle treated and the GPI1046 treated animals were compared. There was a significant difference in the loss of hair cells between vehicle and GPI-1046 treatments (~31%, Figure 5). While neomycin in the vehicle treated animals induced about 75% of hair cell loss, GPI1046 treatment resulted in a loss that was around 45%. The significant protection effects of GPI-1046 was seen on the apex turns and top middle turns (Figure 6).

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CLAIMS

What is claimed is:

1. A method for treating hearing loss comprising administering to a subject a therapeutically effective amount of a neuroimmunophilin compound.
2. The method of claim 1, wherein the hearing loss is associated with injury or degeneration of neuroepithelial hair cells in the inner ear.
3. The method of claim 1, wherein the hearing loss is associated with injury or degeneration of spiral ganglion neurons.
4. The method of claim 1, wherein the neuroimmunophilin compound is a synthetic small molecule.
7. The method of claim 1, wherein the neuroimmunophilin compound is administered at a dose of about 1 ng/kg/day to about 20 mg/kg/day.
11. A method for treating lesions or disturbances to the vestibular apparatus comprising administering to a subject having such a lesion or disturbance a therapeutically effective amount of a neuroimmunophilin compound.
12. The method of claim 11, wherein the lesion or disturbance results in dizziness, vertigo or loss of balance.

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METHOD FOR PREVENTING AND TREATING
HEARING LOSS USING NEUROIMMUNOPHILIN LIGANDS

ABSTRACT

The present invention relates generally to methods for preventing and/or treating injury or degeneration of cochlear hair cells and spiral ganglion neurons by administering immunophilins ligands, and in particular the neuroimmunophilin family of small molecules. The invention relates more specifically to methods for treating sensorineural hearing loss as well as vestibular disorders and tinnitus.

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Protection effect of immunophilins against cisplatin toxicity

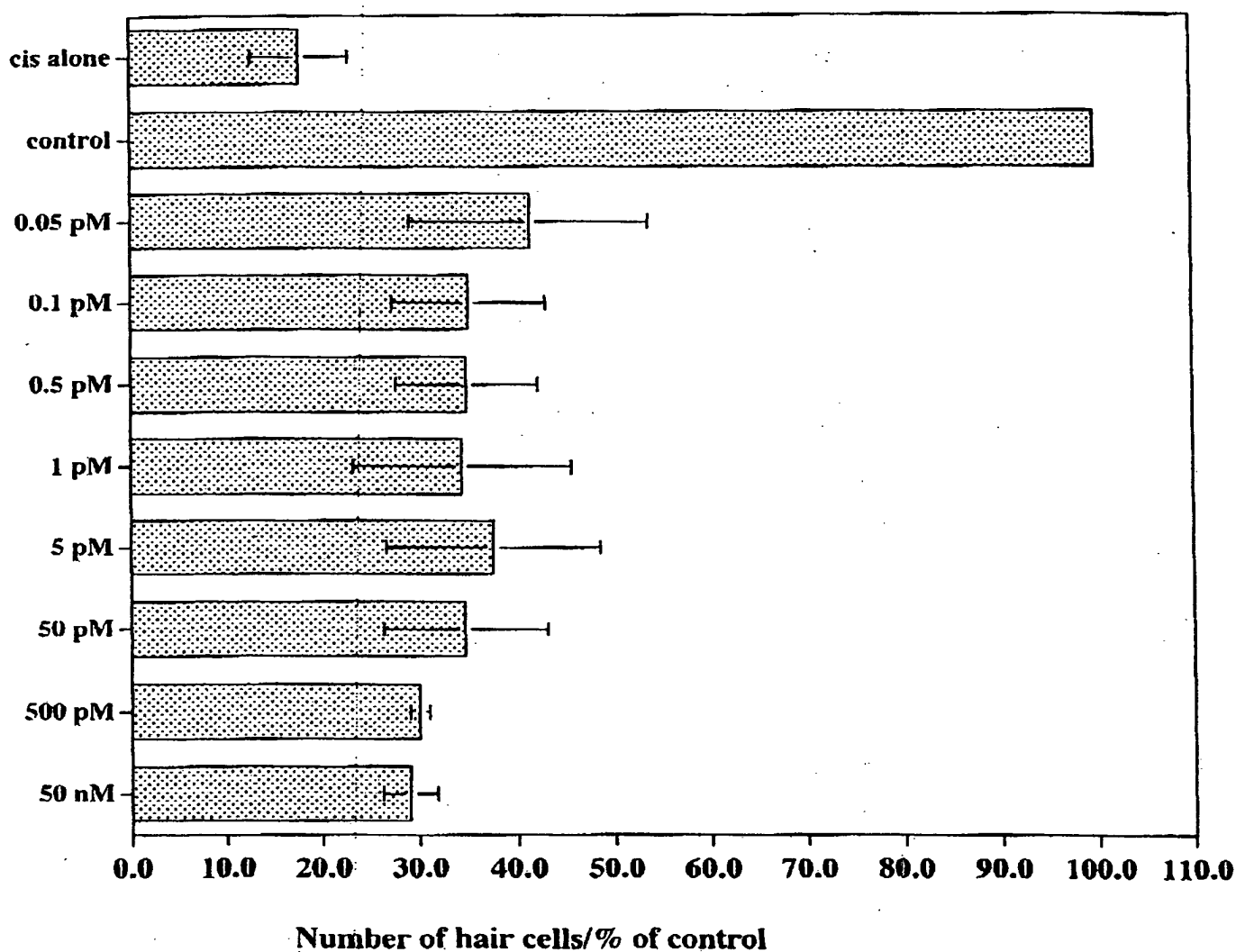


Figure ①

GPI1046 protects hair cells against neomycin toxicity

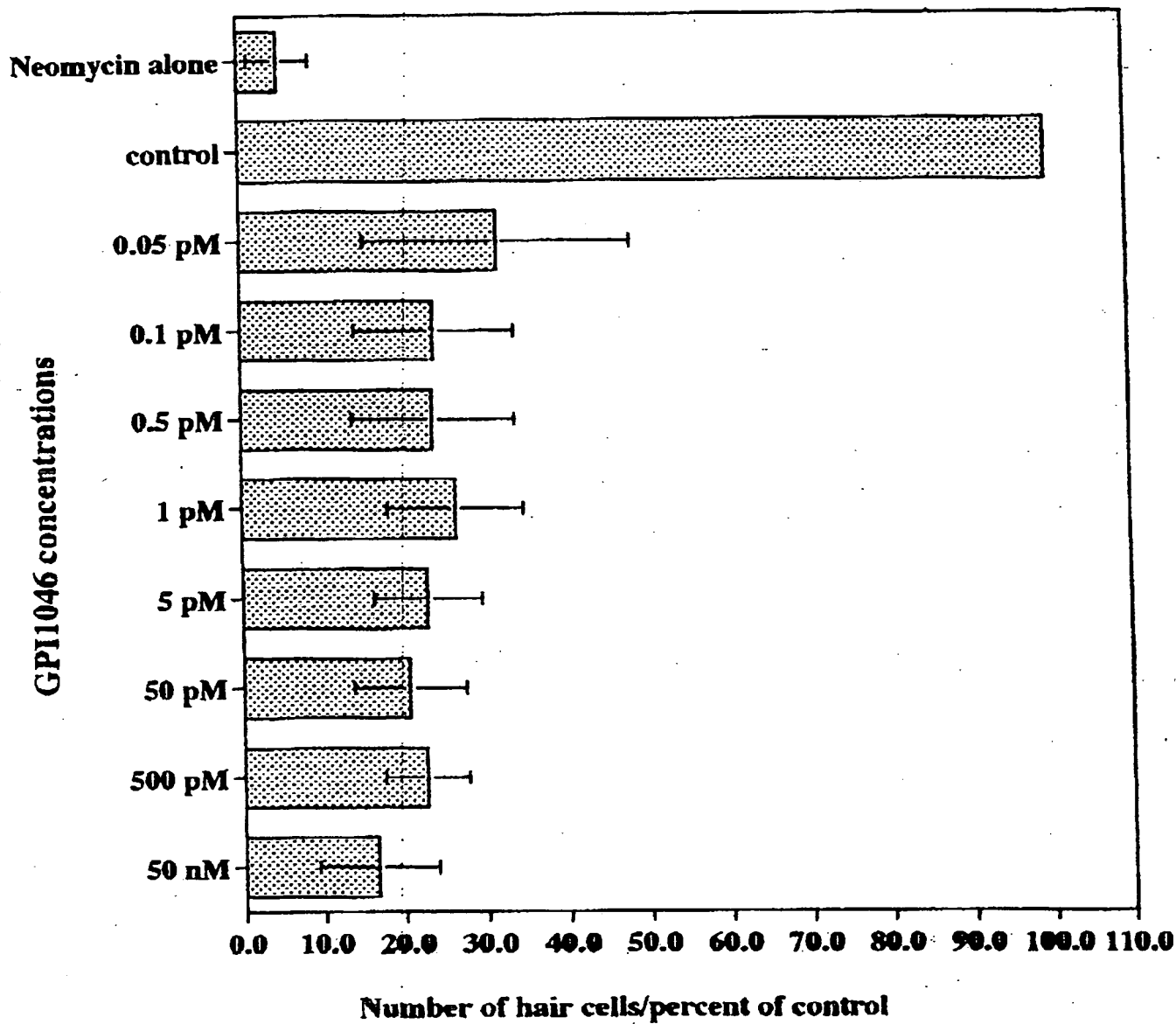
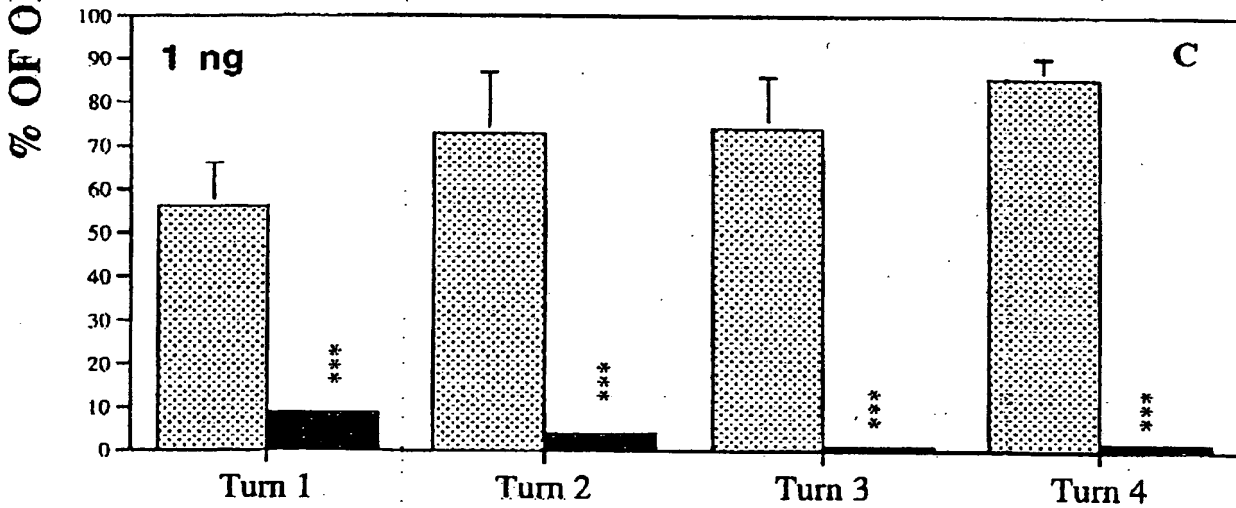
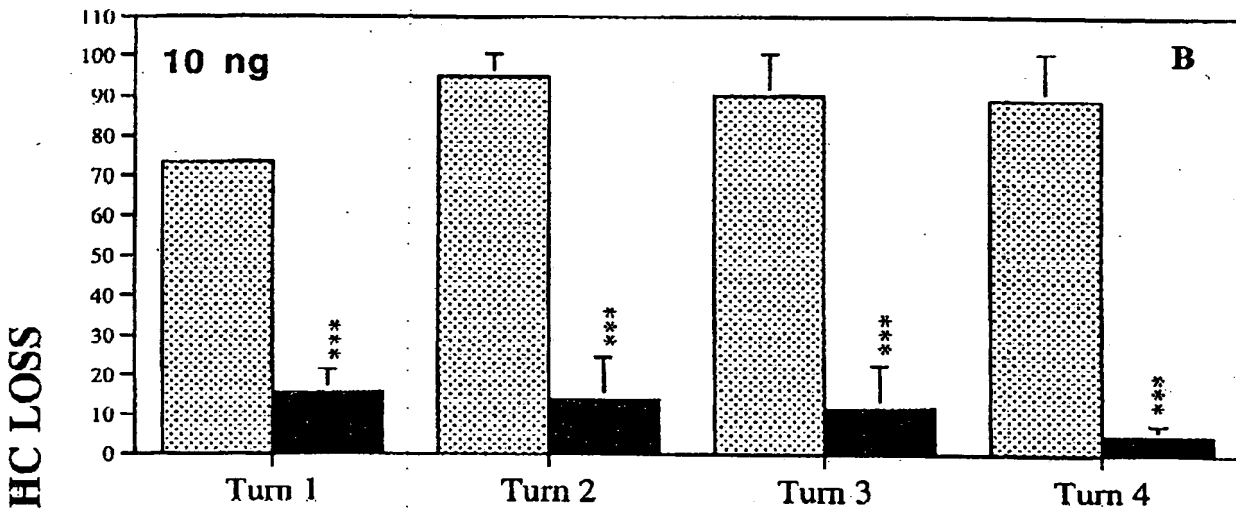
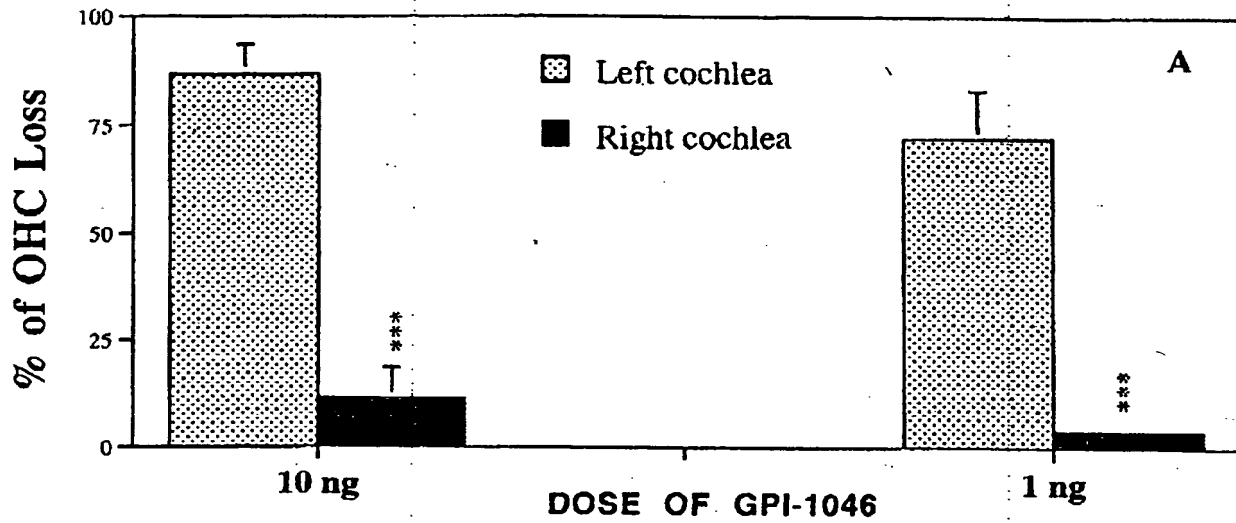
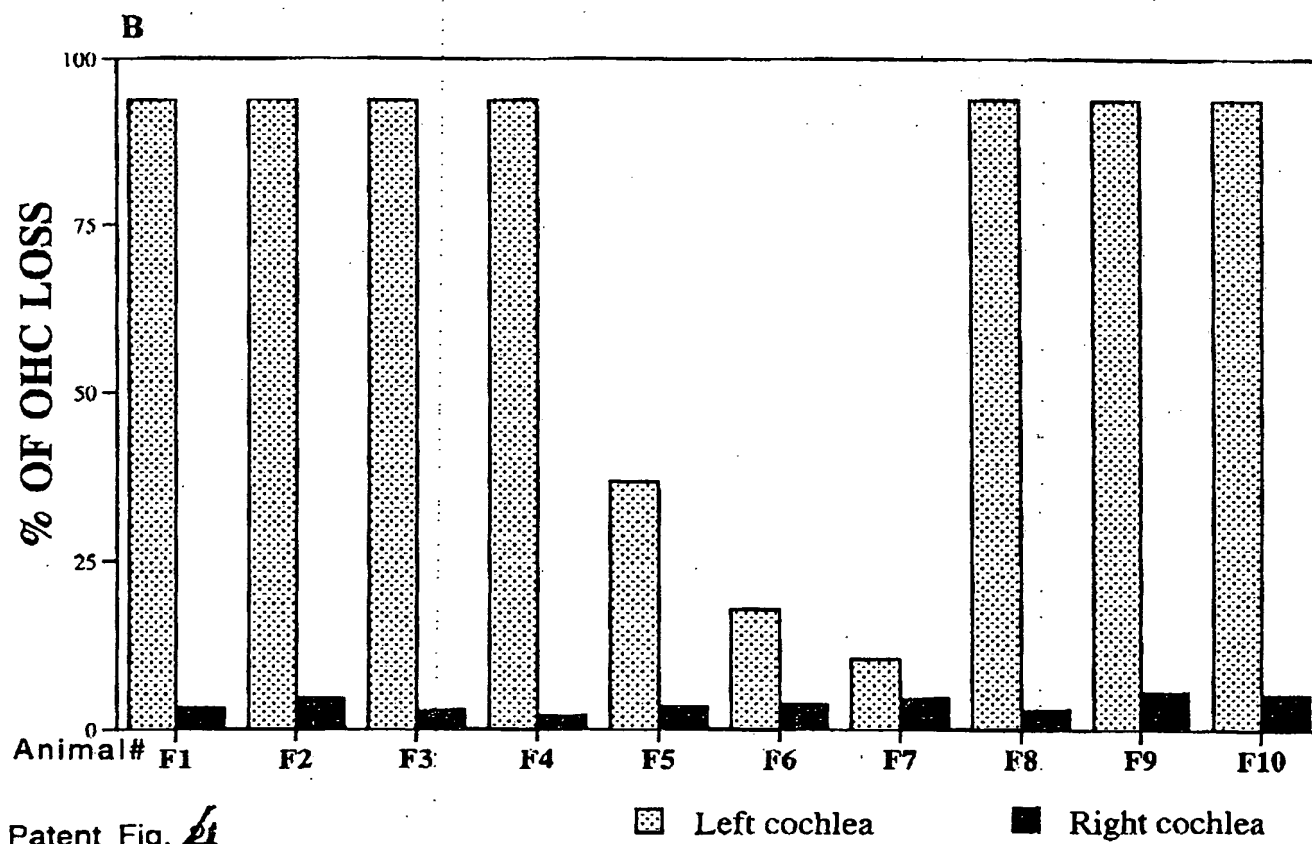
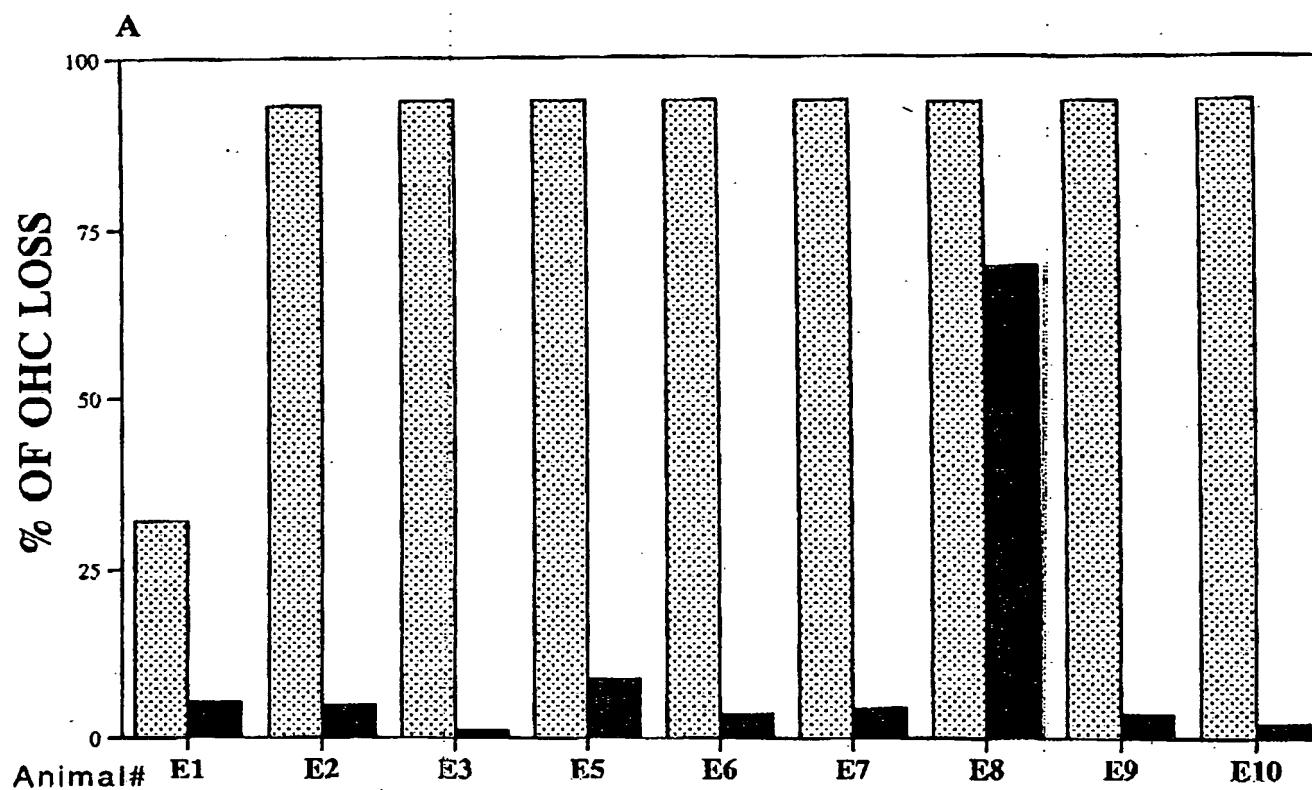


Figure (2)

**COCHLEAR TURNS***** $p < 0.0001$, t-test



Patent Fig. 4

Saline or Neomycin Applied to Round Window,
Vehicle or Fx Administered via S.C. Injection

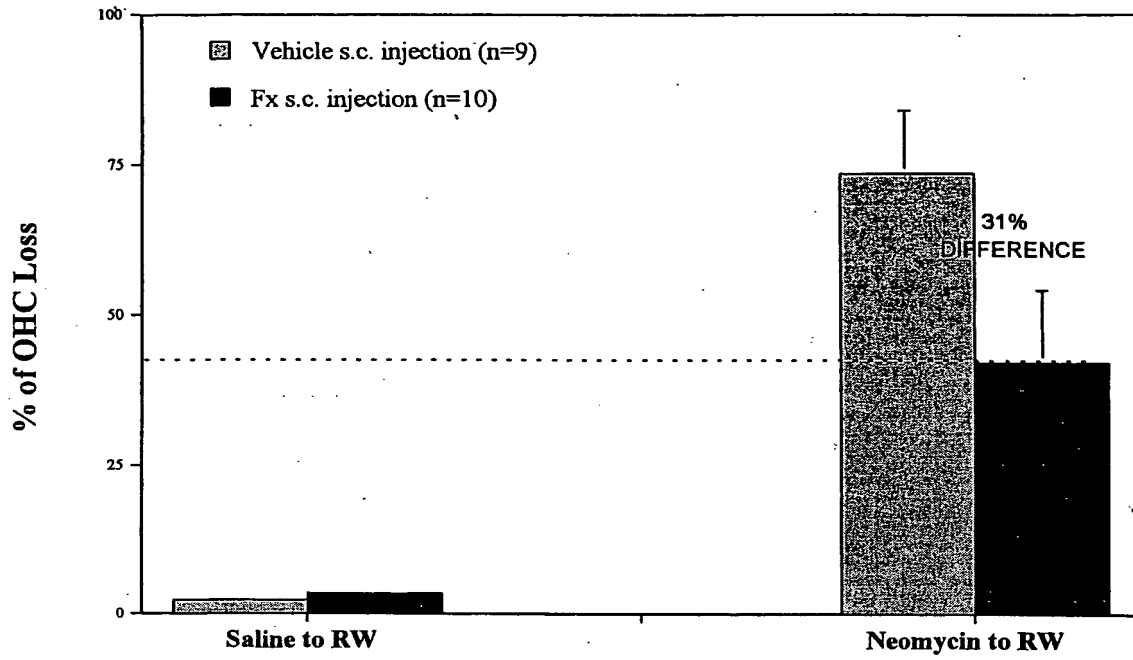


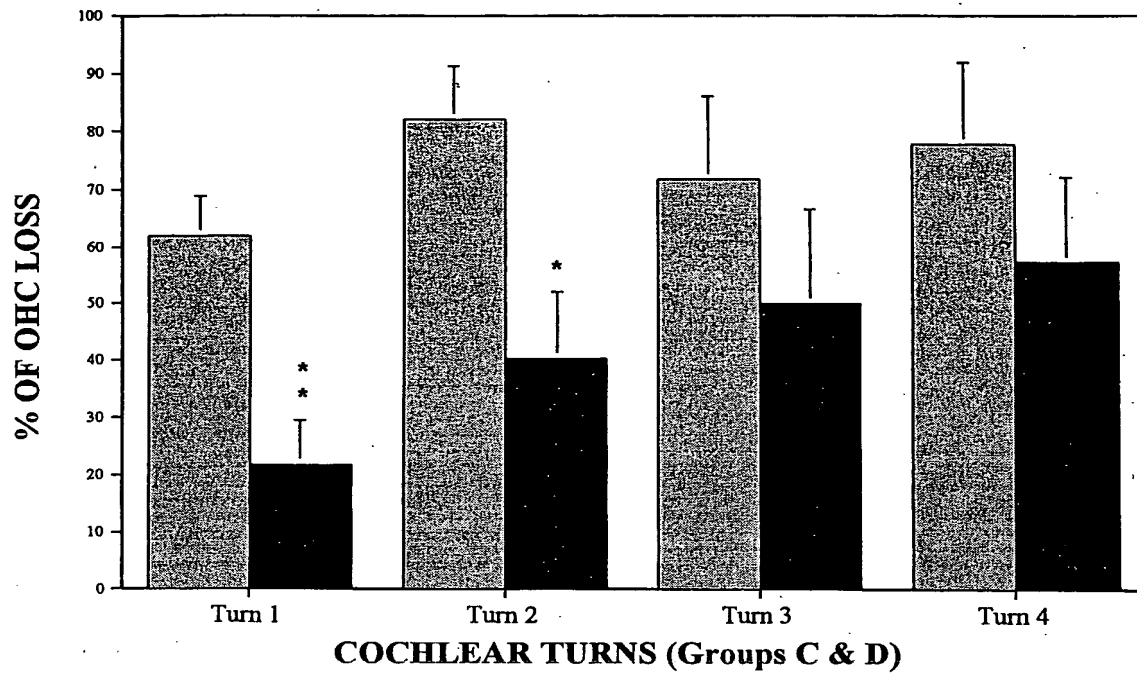
Figure 5

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**Neomycin Applied to Round Window, Vehicle
or Fx Administered via S.C. Injection**

▨ vehicle s.c. injection (n=9)

■ Fx s.c. injection (n=10)



** p<0.01

* p<0.05

t-test

Figure 6

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APPENDIX D



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BOX PROVISIONAL PATENT APPLICATION
Attorney Docket No. 23427

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Ella MAGAL

Serial No. Not Yet Assigned

Filed: September 25, 1997

For: METHODS FOR PREVENTING AND TREATING HEARING LOSS USING
NEUROIMMUNOPHILIN LIGANDSTRANSMITTAL LETTERAssistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith for filing in the U.S. Patent and Trademark
Office is the following:

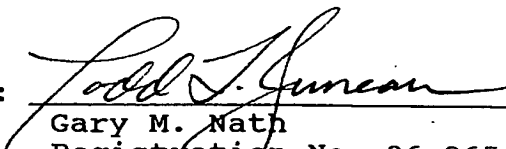
- (1) Transmittal Letter;
- (2) Provisional Application For Patent Cover Sheet;
- (3) Provisional Application consisting of 42 pages including:
 - 34 pages of textual specification
 - 1 page of 7 claims
 - 1 page of the Abstract of the Disclosure
 - 6 sheets of Drawings;
- (4) Authorization for Deposit Acct. 14-0112 in the amount of \$150.00.

Please charge any required fee, or credit any overpayment, in
connection with this matter to deposit Account No. 14-0112.

Respectfully submitted,

Date: September 25, 1997
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09/25/97

PATENT APPLICATION SERIAL NO.

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

12/12/1997 DTHOMAS 00000066 DA#140112 60059963
FC:114 150.00 CH

**METHOD FOR PREVENTING AND TREATING HEARING LOSS USING NEUROIMMUNOPHILIN
LIGANDS.**

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METHOD FOR PREVENTING AND TREATING HEARING LOSS USING NEUROIMMUNOPHILIN LIGANDS.

BACKGROUND OF THE INVENTION

The present invention relates generally to methods for preventing and/or treating injury or degeneration of inner ear sensory cells, such as hair cells and auditory neurons, by administering neuroimmunophilins family products. The invention relates specifically to methods for preventing and/or treating hearing loss due to variety of causes.

INTRODUCTION ABOUT NEUROIMMUNOPHILINS

The neuroepithelial hair cells in the organ of Corti of the inner ear, transduce sound into neural activity, which is transmitted along the cochlear division of the eighth cranial nerve. This nerve consists of fibers from three types of neurons (Spoendlin, H. H. In: Friedmann, I. Ballantyne, J., eds. Ultrastructural Atlas of the Inner Ear; London, Butterworth, pp. 133-164, 1984): 1) afferent neurons, which lie in the spiral ganglion and connect the cochlea to the brainstem. 2) efferent olivocochlear neurons, which originate in the superior olivary complex and 3) autonomic adrenergic neurons, which originate in the cervical sympathetic trunk and innervate the cochlea. In the human, there are approximately 30,000 afferent cochlear neurons, with myelinated axons, each consisting of about 50 lamellae, and 4-6 μm in diameter. This histologic structure forms the basis of uniform conduction velocity, which is an important functional feature. Throughout the length of the auditory nerve, there is a

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trophic arrangement of afferent fibers, with 'basal' fibers wrapped over the centrally placed 'apical' fibers in a twisted rope-like fashion. Spoendlin (Spoendlin, H. H. In: Naunton, R. F., Fernadex, C. eds. *Evoked Electrical Activity in the Auditory Nervous System*. London, Academic Press, pp. 21-39, 1978) identified two types of afferent neurons in the spiral ganglion on the basis of morphologic differences: type I cells (95%) are bipolar and have myelinated cell bodies and axons that project to the inner hair cells. Type II cells (5%) are monopolar with unmyelinated axons and project to the outer hair cells of the organ of Corti. Each inner hair cell is innervated by about 20 fibers, each of which synapses on only one cell. In contrast, each outer hair cell is innervated by approximately six fibers, and each fiber branches to supply approximately 10 cells. Within the cochlea, the fibers divide into: 1) an inner spiral group, which arises primarily ipsilaterally and synapses with the afferent neurons to the inner hair cells, and 2) a more numerous outer radial group, which arises mainly contralaterally and synapses directly with outer hair cells. There is a minimal threshold at one frequency, the characteristic or best frequency, but the threshold rises sharply for frequencies above and below this level (Pickles, J.O. In: *Introduction to the Physiology of Hearing*. London, Academic Press, pp. 71-106, 1982). Single auditory nerve fibers therefore appear to behave as band-pass filters. The basilar membrane vibrates preferentially to different frequencies, at different distances along its length, and the frequency selectivity of each cochlear nerve fiber is similar to that of the inner hair cell to which the fiber is connected. Thus, each cochlear nerve fiber exhibits a turning curve covering a different range of frequencies from its neighboring fiber (Evans, E. F. In: Beagley H. A. ed. *Auditory investigation: The Scientific and Technological basis*. New York, Oxford University Press, 1979). By this mechanism, complex sounds are broken down into component frequencies (frequency resolution) by the filters of the inner ear.

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Hearing loss of a degree sufficient to interfere with social and job-related communications is among the most common chronic neural impairments in the US population. On the basis of health-interview data (Vital and health statistics. Series 10. No. 176. Washington, D.C. (DHHS publication no. (PHS) 90-1504), it is estimated that approximately 4 percent of people under 45 years of age and about 29 percent of those 65 years or over have a handicapping loss of hearing. It has been estimated that more than 28 million Americans have hearing impairment and that as many as 2 million of this group are profoundly deaf (A report of the task force on the National Strategic plan. Bethesda, Md.: National Institute of Health, 1989). The prevalence of hearing loss increases dramatically with age. Approximately 1 per 1000 infants has a hearing loss sufficiently severe to prevent the unaided development of spoken language (Gentile, A. et al. Characteristics of persons with impaired hearing: United States, 1962-1963. Series 10. No. 35. Washington, D.C.: Government printing office, 1967 (DHHS publication no. (PHS) 1000) (Human communication and its disorders: an overview. Bethesda, Md.: National Institutes of health, 1970). More than 360 per 1000 persons over the age of 75 have a handicapping hearing loss (Vital and health statistics. Series 10. No. 176. Washington, D.C. (DHHS publication no. (PHS) 90-1504).

It has been estimated that the cost of lost productivity, special education, and medical treatment may exceed \$30 billion per year for disorders of hearing, speech and language (1990 annual report of the National Deafness and other Communication Disorders Advisory Board. Washington, D.C.: Government Printing Office, 1991. (DHHS publication no. (NIH) 91-3189). The major common causes of profound deafness in childhood are genetic disorders and meningitis, constituting approximately 13 percent and 9 percent of the total, respectively (Hotchkiss, D. Demographic aspects of hearing impairment: questions and answers. 2nd ed. Washington, D.C.: Gallaudet University Press, 1989). In approximately 50 percent of the cases of childhood deafness, the cause is unknown, but is likely due to genetic causes or

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predisposition(Nance WE, Sweeney A. Otolaryngol. Clin. North Am 1975; 8: 19-48).

Impairment anywhere along the auditory pathway, from the external auditory canal to the central nervous system, may result in hearing loss. The auditory apparatus can be subdivided into the external and middle ear, inner ear and auditory nerve and central auditory pathways. Auditory information in humans is transduced from a mechanical signal to a neurally conducted electrical impulse by the action of approximately 15,000 neuroepithelial cells (hair cells) and 30,000 first-order neurons (spiral ganglion cells) in the inner ear. All central fibers of spiral ganglion neurons form synapses in the cochlear nucleus of the pontine brainstem. The number of neurons involved in hearing increases dramatically from the cochlea to the auditory brain stem and the auditory cortex. All auditory information is transduced by only 15,000 hair cells, of which the so-called inner hair cells, numbering 3500, are critically important, since they form synapses with approximately 90 percent of the 30,000 primary auditory neurons. Thus, damage to a relatively few cells in the auditory periphery can lead to substantial hearing loss. Hence, most causes of sensorineural loss can be ascribed to lesions in the inner ear (Nadol, J.B., New England Journal of Medicine, 1993, 329: 1092-1102).

Hearing loss can be on the level of conductivity, sensorineural and central level. Conductive hearing loss is caused by lesions involving the external or middle ear, resulting in the destruction of the normal pathway of airborne sound amplified by the tympanic membrane and the ossicles to the inner ear fluids. Sensorineural hearing loss is caused by lesions of the cochlea or the auditory division of the eight cranial nerve. Central hearing loss is due to lesions of the central auditory pathways. These consist of the cochlear and dorsal olivary nucleus complex, inferior colliculi, medial geniculate bodies, auditory cortex in the temporal lobes and interconnecting afferent and efferent fiber tracts (Adams R. D. and Maurice, V.

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Eds. in: Principles of Neurology. 1989. McGraw-Hill Information services Company. PP 226-246).

As mentioned previously, at least 50 percent of cases of profound deafness in childhood have genetic causes (Brown, K. S. Med. Clin. North AM. 1969; 53: 741-72). If one takes into consideration the probability that genetic predisposition is a major causative factor in presbycusis - or age-related hearing loss- which affects one third of the population over 75 years of age (Nadol, J. B. In: Beasley DS, Davis GA, eds. Aging: Communication Processes and Disorders. New York: Grune & Stratton, 1981:63-85), genetic and hereditary factors are probably the single most common cause of hearing loss. Genetic anomalies are much more commonly expressed as sensorineural hearing loss than as conductive hearing loss. Genetically determined sensorineural hearing loss is clearly a major, if not the main cause of sensorineural loss, particularly in children (Nance WE, Sweeney A. Otolaryngol. Clin. North Am 1975; 8: 19-48). Among the most common syndromal forms of sensorineural loss are Waardenburg's syndrome, Alport's syndrome and Usher's syndrome.

A variety of commonly used drugs have ototoxic properties. The best known are the aminoglycoside antibiotics (Lerner, S. A. et al eds. Aminoglycoside ototoxicity. Boston: Little, Brown, 1981; Smith, C. R. et al. N Engl. J. Med. 1980; 302: 1106-9), loop diuretics (Bosher, S. K., Acta Otolaryngol. (Stockh) 1980; 90: 4-54), salicylates (Myers, E. N. et al. N Engl. J. Med. 1965; 273:587-90) and antineoplastic agents such as cisplatin (Strauss, M. et al. Laryngoscope 1983; 143:1263-5). Ototoxicity has also been described during oral or parenteral administration of erythromycin (Kroboth, P. D. et al. Arch. Intern Med. 1983; 113:169-79; Achweitzer, V. G., Olson, N. Arch. Otolaryngol. 1984; 110:258-60).

Most ototoxic substances cause hearing loss by damaging the cochlea, particularly the auditory hair cells and the stria vascularis, a specialized epithelial organ within the inner ear, that is responsible for the homeostasis of fluids and

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electrolytes (Nadol, J.B. New England J. Med. 1993, 329: 1092-1102). Secondary neural degeneration may occur many years after an ototoxic event affecting the hair cells. There is evidence that some ototoxic substances may be selectively concentrated within the inner ear, resulting in progressive sensorineural loss despite the discontinuation of systemic administration (Federspil, P. et al. J. Infect. Dis. 1976; 134 Suppl: S200-S205).

Trauma due to acoustic overstimulation is another leading cause of deafness. There is individual susceptibility to trauma from noise. Clinically important sensorineural hearing loss may occur in some people exposed to high-intensity noise, even below levels approved by the Occupational Safety and Health Agency. (Osguthorpe, J. D. ed. Washington D.C.: American Academy of Otolaryngology-Head and Neck Surgery Foundation, 1988).

Demyelinating processes, such as multiple sclerosis, may cause sensorineural hearing loss (Noffsinger, D et al. Acta Otolaryngol Suppl (Stockh) 1972; 303:1-63). More recently, a form of immune-mediated sensorineural hearing loss has been recognized (McCabe, B. F. Ann Otol Rhinol Laryngol 1979; 88:585-9). The hearing loss is usually bilateral, is rapidly progressive (measured in weeks and months), and may or may not be associated with vestibular symptoms.

A variety of tumors, both primary and metastatic, can produce either a conductive hearing loss, or a sensorineural hearing loss, by invading the inner ear or auditory nerve (Houck, J. R. et al. Otolaryngol Head Neck Surg 1992; 106:92-7). A variety of degenerative disorders of unknown cause can produce sensorineural hearing loss. Meniere's syndrome (Nadol, J. B. ed. Meniere's disease: pathogenesis, pathophysiology, diagnosis, and treatment. Amsterdam: Kugler & Ghedini 1989), characterized by fluctuating sensorineural hearing loss, episodic vertigo, and tinnitus, appears to be caused by a disorder of fluid homeostasis within the inner ear, although the pathogenesis remains unknown. Sudden idiopathic sensorineural hearing loss (Wilson, W. R. et al. Arch Otolaryngol 1980; 106:772-6), causing moderate-to-severe

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sensorineural deafness, may be due to various causes, including inner ear ischemia and viral labyrinthitis.

Presbycusis, the hearing loss associated with aging, affects more than one third of persons over the age of 75 years. The most common histopathological correlate of presbycusis is the loss of neuroepithelial (hair) cells, neurons, and the stria vascularis of the peripheral auditory system (Schuknecht H. F. Pathology of the Ear. Cambridge, Mass: Harvard University Press, 1974:388-403). Presbycusis is best understood as resulting from the cumulative effects of several noxious influences during life, including noise trauma, ototoxicity and genetically influenced degeneration.

VESTIBULAR DISORDERS - add introduction

TINNITUS - add introduction

SUMMARY OF THE INVENTION

The present invention provides methods for treating sensorineural hearing loss comprising administering to a subject having a lesion in the inner ear a therapeutically effective amount of a neuroimmunophilins family small molecules. For example, the hearing loss may be associated with injury or degeneration of neuroepithelial hair cells (cochlear hair cells) or spiral ganglion neurons in the inner ear.

The present invention is based on the discoveries that hair cells respond to neuroimmunophilins family small molecules (GPI1046) by resisting the toxic effects of ototoxins, such as cisplatin and neomycin. Thus, a therapeutically effective amount neuroimmunophilins family small molecules may be administered to promote the protection, survival or regeneration of hair cells and spiral ganglion neurons.

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It has also been discovered that lesions or disturbances to the vestibular apparatus which sensory transducing organ consists of the same hair cells as the cochlea, may also be treated by administering to a subject having such a lesion or disturbance a therapeutically effective amount of a neuroimmunophilins family small molecules. Such lesions may result in dizziness, vertigo or loss of balance.

According to the invention, the neuroimmunophilins family small molecules may be administered parenterally at a dose ranging from about 1 ng/ear/day to about 10 ng/ear/day, typically at a dose of about 1 μ g/ear/day to about 10 μ g/ear/day, and usually at a dose of about 5 mg/kg/day to about 20 mg/kg/day. It is also contemplated that, depending on the individual patient's needs and route of administration, the neuroimmunophilins family small molecules may be given at a lower frequency such as monthly, weekly or several times per week, rather than daily. It is further contemplated that neuroimmunophilins family small molecules may be administered directly into the middle ear or the inner ear. One skilled in the art will appreciate that with such administration of a smaller amount of neuroimmunophilins family small molecules may be used, for example, a direct middle ear or inner-ear administration dose in the range of about 1 ng/ear to about 10 ng/ear in a single injection or in multiple injections.

It is further contemplated that neuroimmunophilins family small molecules be administered in combination or conjunction with an effective amount of a second therapeutic agents, such as GDNF, BDNF and NT-3. The invention also provides for the use of neuroimmunophilins family small molecules in the manufacture of a medicament or pharmaceutical composition for the treatment of injury or degeneration of hair cells and auditory neurons for the variety of causes of sensorineural hearing loss. Such pharmaceutical compositions include topical, oral or middle and inner ear neuroimmunophilins family small molecules formulations or in combination with cochlear implants.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for preventing and/or treating sensorineural hearing loss by administering a therapeutically effective amount of neuroimmunophilins family small molecules. According to one aspect of the invention, methods are provided for treating damaged hair cells and auditory neurons by administering a therapeutically effective amount of neuroimmunophilins family small molecules by means of a pharmaceutical composition. The invention may be practiced using any biologically active neuroimmunophilins family small molecules.

The present invention is based on the initial discoveries that neuroimmunophilins family small molecules protect hair cells from ototoxins-induced cell death in explant cultures of rat's cochlea and in animal model (guinea pig) of deafness. It is contemplated that administration of exogenous neuroimmunophilins family small molecules will protect hair cells and spiral ganglion neurons from traumatic damage (such as noise trauma and acute or chronic treatments of cisplatin and aminoglycoside antibiotics) or from damage resulting from a lack of neurotrophic factors caused by interruption of transport of the factors from the axon to the cell body. Such treatment is expected to allow hair cells and /or auditory neurons to tolerate intermittent insults from either environmental noise trauma, treatments with ototoxins and to slow down, prevent or reverse the progressive degeneration of the auditory neurons and hair cells, that is responsible for hearing loss in pathological conditions such as presbycusis (age-related hearing loss), inherited sensorineural degeneration, and post-idiopathic hearing losses and to preserve the functional integrity of the inner ear. It will also support

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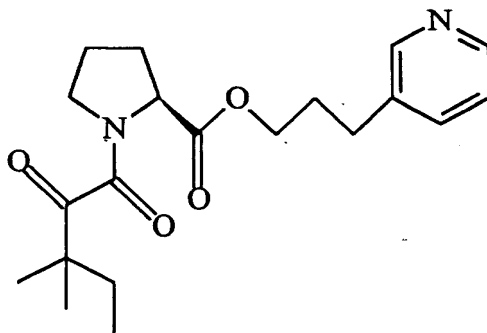
the auditory neurons for a better and longer performance of cochlear implants.

According to the invention, the neuroimmunophilins family small molecules may be administered into the middle ear at a dose ranging from about 1 ng/ear/day to about 10 ng/ear/day, typically at a dose of about 1 µg ear/day to about 10 µg/ear/day, and usually at a dose of about 5 µg/ear/day to about 20 µg/ear/day. GDNF protein neuroimmunophilins family small molecules may be administered directly into the inner ear in cases where invasion of the inner ear is already in place such as in the procedure of cochlear implant or surgeries of the inner ear. In such cases, a smaller amount of neuroimmunophilins family small molecules will be administered, for example, from about 0.1 ng/ear to about 1 ng/ear in a single injection or in multiple injections.

Neuroimmunophilins family small molecules can be also developed in a form of ear-drops which will penetrate the tympanic membrane of the Bulla. It is further contemplated that neuroimmunophilins family small molecules be administered with an effective amount of a second therapeutic agent for the treatment of auditory neuron degeneration, together with GDNF, BDNF and NT-3 as well as other factors or drugs used currently or in the future for the treatment of the various inner ear pathologies. A variety of pharmaceutical formulations and different delivery techniques are described in further detail below.

As used herein, the term ~~⌘~~ GPI1046 ~~⌘~~ refers to the compound 3-(3-pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate, exemplified in Steiner et al., Proc. Natl. Acad. Sci., Vol. 94, pp.2019-2024, March, 1997, and in particular, Scheme I on page 2021, the entire contents of which are incorporated by reference herein. The chemical structure of GPI 1046 is provided in Steiner et al. and reproduced below in Formula I.

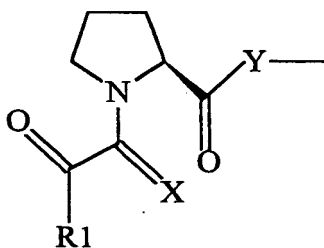
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I

3-(3-pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate

As used herein, the term "neuroimmunophilins family small molecules" includes all biologically active synthetic compounds that bind to the protein receptor FKBP12 and chemically modified derivatives thereof. Particularly preferred compounds are described in U.S. Patent No. 5,614,547, issued March 25, 1997 to Hamilton et al., the entire contents of which are incorporated herein by reference, and which are described by Formula II below, wherein Y is O or NR₂, X is O or S, and R₁, R₂, and Z are independently any chemically reasonable substituent.



II

The term "biologically active" as used herein means that the neuroimmunophilins family small molecules demonstrates similar neurotrophic properties, but not necessarily all of the same

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properties, and not necessarily to the same degree, as the GPI1046.

Neuroimmunophilins family small molecules may also be chemically synthesized by any means known to those skilled in the art, and particular synthetic schemes are exemplified in U.S. Patent No. 5,614,547 to Hamilton.

C. Neuroimmunophilins family small molecules Pharmaceutical Compositions

Neuroimmunophilins family small molecules pharmaceutical compositions typically include a therapeutically effective amount of neuroimmunophilins family small molecules in admixture with one or more pharmaceutically and physiologically acceptable formulation materials. Suitable formulation materials include, but are not limited to, antioxidants, preservatives, coloring, flavoring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. For example, a suitable vehicle may be water for injection, physiological saline solution, or artificial perilymph, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles.

The primary solvent in a vehicle may be either aqueous or non-aqueous in nature. In addition, the vehicle may contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the vehicle may contain still other pharmaceutically-acceptable excipients for modifying or maintaining the rate of release of GDNF protein product, or for promoting the absorption or penetration of GDNF protein product across the tympanic membrane. Such excipients are those substances usually and customarily employed to formulate dosages.

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for middle-ear administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or in a form, e.g., lyophilized, requiring reconstitution prior to administration.

The optimal pharmaceutical formulations will be determined by one skilled in the art depending upon considerations such as the route of administration and desired dosage. See for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712, the disclosure of which is hereby incorporated by reference. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the present GDNF proteins, variants and derivatives.

Other effective administration forms, such as middle-ear slow-release formulations, inhalant mists, or orally active formulations are also envisioned. For example, in a sustained release formulation, the neuroimmunophilins family small molecules may be bound to or incorporated into particulate preparations of polymeric compounds (such as polylactic acid, polyglycolic acid, etc.) or liposomes. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. The neuroimmunophilins family small molecules pharmaceutical composition also may be formulated for middle-ear administration, e.g., by tympanic membrane infusion or injection, and may also include slow-release or sustained circulation formulations. Such middle-ear administered therapeutic compositions are typically in the form of a pyrogen-free, middle-ear acceptable aqueous solution comprising the neuroimmunophilins family small molecules in a pharmaceutically acceptable vehicle. One preferred vehicle is sterile distilled water.

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It is also contemplated that certain formulations containing neuroimmunophilins family small molecules are to be administered orally. neuroimmunophilins family small molecules which is administered in this fashion may be encapsulated and may be formulated with or without those carriers customarily used in the compounding of solid dosage forms. The capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional excipients may be included to facilitate absorption of neuroimmunophilins family small molecules. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

The formulation of topical ear preparations, including middle-ear solutions, suspensions and ointments is well known to those skilled in the art (see Remington's Pharmaceutical Sciences, 18th Edition, Chapter 86, pages 1581-1592, Mack Publishing Company, 1990). Other modes of administration are available, including injections to the middle ear, and methods and means for producing middle-ear preparations suitable for such modes of administration are also well known.

As used in this application, "middle-ear" refers to the space between the tympanic membrane and the inner ear. This location is external to all inner ear tissue and an invasive procedure might not be required to access this region if a formulation will be developed so that the neuroimmunophilins family small molecules will penetrate through the tympanic membrane. Otherwise, the material will be introduced to the middle ear by injection through the tympanic membrane or, in case repeated administrations are needed, a hole will be made in the tympanic membrane. Examples of such systems include inserts and "topically" applied drops, gels or ointments which may be used to deliver therapeutic material to these regions. An opening in the tympanic membrane is a very frequent procedure done on a office-visit basis, in cases such as infections of the middle ear

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(usually in children). The opening closes spontaneously after a few days.

In the presently described use of neuroimmunophilins family small molecules of the treatment of inner ear disease or injury it is also advantageous that a topically applied formulation include an agent to promote the penetration or transport of the therapeutic agent into the middle and inner ear. Such agents are known in the art. For example, Ke et al., U.S. 5,221,696 disclose the use of materials to enhance the penetration of ophthalmic preparations through the cornea.

Inner-ear systems are those systems which are suitable for use in any tissue compartment within, between or around the tissue layers of the inner-ear, such as the cochlea and vestibular organ. These locations include the different structures of the cochlea such as the stria vascularis, Reissner's membrane, organ of Corti, spiral ligament and the cochlear neurons. An invasive procedure might not be required to access those structures since it has been shown that even proteins, let alone small molecules, do penetrate the membrane of the round window into the perilymph of the inner ear. A particularly suitable vehicle for introducing neuroimmunophilins family small molecules into the inner ear by penetration through the round window membrane is artificial perilymph. This solution consists of 10.00 mM D-glucose, 1.5 mM CaCl₂, 1.5 mM MgCl₂ in a 1.0% solution of Dulbecco's phosphate-buffered saline in deionized water at 280-300 mOsm and pH of 7.2. Yet another preparation may involve the formulation of the neuroimmunophilins family small molecules with an agent, such as injectable microspheres or liposomes into the middle ear, that provides for the slow or sustained release of the molecules which may then be delivered as a depot injection. Other suitable means for the inner-ear introduction of neuroimmunophilins family small molecules includes, implantable drug delivery devices or which contain the neuroimmunophilins family small molecules, and a cochlear-implant with a tunnel through, so neuroimmunophilins family small

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molecules can be continuously delivered through it to the inner ear.

The ear-treatment preparations of the present invention, particularly topical preparations, may include other components, for example middle-ear acceptable preservatives, tonicity agents, cosolvents, complexing agents, buffering agents, antimicrobials, antioxidants and surfactants, as are well known in the art. For example, suitable tonicity enhancing agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol and the like. Sufficient tonicity enhancing agent is advantageously added so that the formulation to be instilled into the ear is compatible with the osmolarity of the endo- and perilymph. Suitable preservatives include, but are not limited to, benzalkonium chloride, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and the like. Hydrogen peroxide may also be used as preservative. Suitable cosolvents include, but are not limited to, glycerin, propylene glycol and polyethylene glycol. Suitable complexing agents include caffeine, polyvinylpyrrolidone, beta -cyclodextrin or hydroxypropyl- beta -cyclodextrin. The buffers can be conventional buffers such as borate, citrate, phosphate, bicarbonate, or Tris-HCl.

The formulation components are present in concentration that are acceptable to the middle or inner ear site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

Additional formulation components may include materials which provide for the prolonged the residence of the middle ear administered therapeutic agent so as to maximize the topical contact and promote absorption through the round window membrane. Suitable materials include polymers or gel forming materials which provide for increased viscosity of the middle-ear preparation. The suitability of the formulations of the instant invention for controlled release (e.g., sustained and prolonged delivery) of an inner-ear treating agent can be determined by

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various procedures known in the art. Yet another ear preparation may involve an effective quantity of neuroimmunophilins family small molecules in a mixture with non-toxic middle-ear treatment acceptable excipients which are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, middle-ear treatment solutions can be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia.

Administration/Delivery of neuroimmunophilins family small molecules

The neuroimmunophilins family small molecules may be administered parenterally via a subcutaneous, intramuscular, intravenous, transpulmonary, transdermal, intrathecal or intracerebral route. For the treatment of inner-ear conditions, the neuroimmunophilins family small molecules may be administered into the middle-ear (or directly into the inner-ear, especially in cases where invasive procedure is already in place), by topical application, inserts, injection and implants. For example, slow-releasing implants containing the molecules embedded in a biodegradable polymer matrix can deliver neuroimmunophilins family small molecules.. Neuroimmunophilins family small molecules may be administered in the middle or inner ear, or it may be administered on top of the tympanic membrane in connection with one or more agents capable of promoting penetration or transport of neuroimmunophilins family small molecules across the membranes of the ear. The frequency of dosing will depend on the pharmacokinetic parameters of the neuroimmunophilins family small molecules as formulated, and the route of administration.

The specific dose may be calculated according to considerations of body weight, body surface area or organ size. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above

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mentioned formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed, especially in light of the dosage information and assays disclosed herein. Appropriate dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data. It will be appreciated by those skilled in the art that the dosage used in inner-ear administered formulations will be minuscule as compared to that used in a systemic injection or oral administration.

The final dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g., the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding the appropriate dosage levels for the treatment of various diseases and conditions.

It is envisioned that the continuous administration or sustained delivery of neuroimmunophilins family small molecules may be advantageous for a given treatment. While continuous administration may be accomplished via a mechanical means, such as with an infusion pump, it is contemplated that other modes of continuous or near continuous administration may be practiced. For example, subcutaneous and muscular injections as well as oral pills and ear drops.

Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible particles or beads and depot injections, are also known to those skilled in the art.

It should be noted that the neuroimmunophilins family small molecules formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the

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case of veterinary applications, the dosage ranges should be the same as specified above.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples. Example 1 and 2 addresses the effects of neuroimmunophilins family small molecules (GPI1046) administration on hair cells in a Cochlear explant culture system. Example 3 and 4 addresses the effects of neuroimmunophilins family small molecules administration on hair cells in cochlea of guinea pigs treated with various clinically relevant ototoxins such as neomycin and cisplatin. The results of the organ of Corti explant cultures studies and that of the animal model of deafness demonstrated that neuroimmunophilins family small molecules has protective activity for the hair cells of the organ of Corti against ototoxins, which were not previously known to be neuroimmunophilins family small molecules responsive.

EXAMPLES

EXAMPLE 1

GPI1046 Protects Cochlear Hair Cells Against Ototoxicity induced by neomycin and cisplatin - In Vitro

MATERIALS

The materials used in the following Example were obtained as follows:

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Organ of Corti dissecting solution:

Dulbecco's Phosphate Buffered Saline (PBS; 1x, without calcium chloride, without magnesium chloride. Cat. #14190-136, Gibco BRL), containing 1.5 g/L D-Glucose (Dextrose. Cat. #15023-021, Gibco BRL).

Organ of Corti explant culture Medium

1. High glucose Dulbecco's Modified Eagle Medium (DMEM; 1 X , with L-glutamine, without Sodium Pyruvate. Cat. #11965-084, Gibco BRL)
2. 0.15 g/100 ml of D-Glucose (Dextrose. Cat. #15023-021, Gibco BRL)
3. 1% N-2 Supplement (100 X, Cat. #17502-030, Gibco BRL)
4. 100 Units/ml of Penicillin G, Potassium (Penicillin; Cat. #21840-020, Gibco BRL)

METHODSPreparation of Medium

DMEM was supplemented with 1 % N-2 supplement, and D-glucose was added to a final concentration of 1.5 g/L. Penicillin was added at 100 Units/ml. After mixing, the medium was filtered and kept at 4°C. The medium was prepared fresh just before use in order to minimize inter-experimental variations. Plastic pipettes and containers were used throughout to minimize protein adsorption.

Dissecting tools and culture dishes

1. The 4" and 5" dissecting forceps and 4" dissecting scissors were from Roboz Surgical, Washington, DC.
2. Falcon sterile 96-well microplates (Flat Bottom. Cat. #3072), tissue culture plastic ware and polypropylene centrifuge tubes were from Beckton-Dickinson, Lincoln Park, New Jersey.

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GPI1046 Product Solutions

GPI1046 stock was stored in room temperature and prepared fresh for each culture. GPI1046 stock solution was diluted in 10 μ l of 100% EtOH for every milligram of GPI1046 stock (approximately 250mM). This solution of 250mM GPI 046 in 100% EtOH was diluted in normal culture medium to working concentrations of 50000 nM, 5000nM, 500nM, 50nM, 5000pM, 500pM, 50pM, 10pM, 5pM, 1pM, 0.5pM, 0.1pM, and 0.01pM. Ten microliters of ten-fold concentrated GPI 1046 product solutions were added to Organ of Corti explant cultures containing ototoxin medium (90 μ l), so that the final GPI 1046 concentration were 5000nM, 500nM, 50nM, 5nM, 500pM, 50pM, 5pM, 1pM, 0.5pM, 0.1pM, 0.05pM, 0.01pM, and 0.001pM. Control cultures received normal medium (10 μ l). The GPI1046 product treatments were initiated at first day culture (one day before ototoxin treatment), and repeated with ototoxin treatment at second day.

Ototoxins and Related Reagents

1. Neomycin solution (Cat. #N1142, Sigma. St. Louis, MO), used at final concentration of 0.6 mM (A fresh solution was made each experiment by adding 90 μ l of 1mg/ml neomycin and to 1410 μ l medium).
2. Cisplatin (Platinol-AQ. Cat. #NDC 0015-3220-22, Bristol-Myers Squibb Laboratories, Princeton, New Jersey). Used at a final concentration of 35 μ g/ml (a fresh solution was prepared each experiment by adding 52.5 μ l of 1 mg/ml cisplatin to 1447.5 μ l medium)
3. Triton X-100 (t-Octylphenoxypoly-ethoxyethanol. Cat. #X-100, Sigma. St. Louis, MO)
4. Phalloidin (FITC Labeled. Cat. #P-5282, Sigma. St. Louis, MO)
5. Vectashield (Mounting Medium, Cat. #H-1000, Vector, Burlingame, CA)

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Preparation of Rat Organ of Corti explant

Organ of Corti explants were obtained from P3-P4 Wistar rats. Rats were decapitated, the lower jaw was cut out and skin removed. The temporal bone was collected in dissection solution, the otic capsule exposed and the bony-cartilaginous cochlear capsule was carefully separated from the temporal bone. Freed cochlea were transferred to another Petri dish with dissection solution for further dissection. Intact organs of Corti were obtained by using a fine forceps to hold central VIII nerve tissue and remove it out, then the stria vascular membrane was carefully stripped off, starting from the apex or base. The organ of Corti then was then transferred to a 35-mm diameter Petri dish containing cold PBS supplemented with glucose and ready to be cultured.

Cochlea explant culture procedure

Cochlea explants were cultured in uncoated 96 microplates. A single organ of Corti was placed in a well and was kept floating in the medium. Explants were kept in normal medium for 24 hours (90 μ l/well). GPI1046 solution (10 μ l) was added to the 'treated' cultures and 10 μ l medium was added to controls. After 24 hours of incubation, the media were changed and the explants were exposed to ototoxin-containing medium (90 μ l), with GPI1046 solution (10 μ l) or without (control). The cultures were incubated for an additional 3 days. The explants were then fixed with 4 % paraformaldehyde in 0.1 M D-PBS for 30 minutes at room temperature and processed for immunostaining.

FITC-phalloidin staining of hair cells

To identify and count hair cells in the organ of Corti, a direct immunostaining method was used to label the actin present naturally in the stereocilia bundles

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of the hair cells. The explants were washed three times with D-PBS (200 μ l per well) and permeabilized with 1 % Triton X-100 in D-PBS for 15 minutes at room temperature. After three washes in D-PBS, the explants were incubated with FITC-labeled Phalloidin (1:60 from stock, 50 μ l/well) for 45 minutes at room temperature. The plates were covered with aluminum foil as the Phalloidin is light sensitive. After three more washes with D-PBS, the labeled explants were placed in a drop of glycerol on a microscope slide, covered with a glass coverslip and sealed with nail polish. The explants were observed under a Nikon Diaphot-300 inverted fluorescence microscope, using FITC filters and fluorescence optics.

Determination of hair cell number

For each experimental point, 2 to 4 cochlea were used. In each cochlea, the number of hair cells was counted in 2-3 section, 175 μ m in length each. Only the sections in the middle turn of the cochlea were analyzed. Each experiment was repeated several times. The numbers of hair cells in control and cisplatin- or neomycin-treated cultures was generated from analyzing 40 cochlea per point.

RESULTS

Hair cells in the floating explant cultures did not die during the experiment period of four days. Thus, the number of phalloidin-stained cells present at the end of the 4 days experiment period, in the absence of ototoxins and treatments, was 105.4 ± 6.9 (n=28). Ototoxins added to the explants on the second day post-plating caused a very significant loss in hair cell number found after 4 days *in vitro*. Exposure to 35 μ g/ml cisplatin 24 hours after plating caused a loss of

more than 80 percent of the hair cells: only $17.6 \% \pm 5.1$ (n=20) of the initial number of hair cells survived (Figure 1) and after exposure to 0.6 mM neomycin, only $5.0\% \pm 3.8$ (n=26) of the hair cells survived (Figure 2).

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There was a marked difference in the morphology of the organs of Corti between this two treatments: while the treatment with neomycin resulted in almost complete loss of hair cells, those that were spared were still organized in the typical four row structure (3 rows of outer hair cells and one row of inner hair cells). Cisplatin treatment, on the other hand, caused a marked disruption of the four-row-structure and the surviving cells were randomly located, indicating a damage caused also to the supporting cells underlying the hair cells.

In cultures that received GPI1046 at the time of plating (pretreatment), a significant number of hair cells survived the 3-day exposure to ototoxins (from day 2 to day 4). In cultures exposed to cisplatin, treatment with GPI1046 at concentrations as low as 0.05 pM resulted in an increase in surviving hair cells from the 17% of the untreated to 41.4%. This, however, was already the maximal activity of GPI1046 as the effect did not titrate out along the range of concentration tested (0.05 pM - 50 nM). Cultures that received neomycin showed reduction of 95% in hair cells compared to controls. Treatment with GPI1046 together with the neomycin reduced this loss to around 70% ($31.8\% \pm 16.4$ surviving hair cells) at a concentration of 0.05 pM, an effect which again did not titrate out nor was increased with higher concentrations of GPI1046.

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EXAMPLE 2

Intramiddle Ear Administered GPI1046 Prot cts Hair
Cells Against intramiddle Ear Neomycin-induced
Ototoxicity

MATERIALS

The materials used in the following Example were obtained as follows:

Ototoxins - Neomycin sulfate: (Cat. #N-1876, Sigma, St. Louis, MO)

immunophilin ligand - GPI1046: supplied by Guilford.

Vehicle - 20% Intralipid: Intralipid is a 20% I.V. fat emulsion (Cat. #NDC 0338-0491-02, Pharmacia Inc., Clayton, NC). Each 100 ml contains: Soybean oil 20.0 g, Phospholipids (from powdered egg yolk) 1.2 g, Glycerin, USP 2.25 g, Water for injection qs, and Calories 200 kcal. pH 8.0 (6.0-8.9), adjusted with sodium hydroxide.

Ethyl alcohol: 200 proof Dehydrated alcohol, USP (Quantum Chemical Company, Tuscola, IL)

Saline solution: 0.9% sterile sodium chloride aqueous solution (Cat #NDC 57319-077-06, Phoenix Pharmaceutical, Inc., St. Joseph, Missouri)

Gelfoam: absorbable gelatin sponge, USP (Cat. #NDC 0009-0396-01, Upjohn, Kalamazoo, MI)

Guinea pigs: Female pigmented guinea pigs (more sensitive than albino to the ototoxicity induced by aminoglycoside antibiotics) from NIH, body weight: 300-400 g

Phalloidin: FITC Labeled. (Cat. #P-5282, Sigma. St. Louis, MO)

Vectashield: Mounting Medium. (Cat. #H-1000, Vector, Burlingame, CA)

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METHODS

1. The First Middle Ear Administration of GPI1046:

20 Guinea pigs used in this study were divided into two groups: 10 animals received 10 ng and 10 received 1 ng of GPI1046.

Preparation of GPI1046: On the day of compound use, GPI1046 stock solutions were prepared as following:

GPI1046 stock a: a stock solution of GPI1046 at 1 mg/10 ml in 100% ethanol was firstly prepared and then it was diluted and mixed in Intralipid at 10 ng/100 μ l.

GPI1046 stock b: a stock solution of GPI1046 at 1 mg/100 ml in 100% ethanol was firstly prepared and then it was diluted and mixed in 20% Intralipid at 1 ng/100 μ l.

Above stock solutions were made fresh daily, and discard after use.

The vehicle was 20% Intralipid.

The Middle Ear Administration:

Animals were anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and xylazine (4 mg/kg). Through a post-auricular incision, the right bulla was identified. A hole was drilled to open the middle ear cavity (care was taken not to injure the tympanic annulus or ossicles). A piece of gelfoam (~2mm³) was soaked with GPI1046 solution and was inserted into the round window niche. The remain GPI1046 solution (~100 μ l) was then injected into the middle ear cavity. In the 10 ng dose group, 100 μ l of GPI1046 stock a was administered; and in the 1 ng dose group, 100 μ l of GPI1046 stock b was administered to the middle ear cavity. The hole was covered with a piece of clear plastic sheet which was stuck on the skull with a superglue. The incision was closed with clippers. The same procedure was performed at the left bulla, but

administered with 100 μ l of vehicle solution instead of GPI1046. The animals were maintained in the prone position until they woke up to verify filling of the middle ear cavity.

2. The Second Middle Ear Administration of GPI1046 and Neomycin Ototoxin:

After two days, the animals received the second administration of GPI1046 or vehicle together with neomycin in the middle ears. Solutions were prepared for above two groups of animals as following:

Solution a: Neomycin was dissolved in GPI1046 stock a described above. The final concentration of neomycin was 5 mg and the GPI1046 was 10 ng in a 100 μ l vehicle solution.

Solution b: Neomycin was dissolved in GPI1046 stock b described above. The final concentration of neomycin was 5 mg and the GPI1046 was 1 ng in a 100 μ l vehicle solution.

Solution c: Neomycin was dissolved in 20% Intralipids to final concentration of 5 mg in 100 μ l vehicle.

When the incision was reopened, the plastic cover sheet on the bulla window was pilled off. The old GPI1046 or vehicle were sucked off and the old gelfoam was removed from the round window niche. A piece of gelfoam with fresh stock solution containing GPI1046 and neomycin was administered to the round window niche, and the remaining solution (~100 μ l, solution a for the 10 ng dose group and solution b for the 1 ng dose group respectively) was injected to the middle ear cavity of the right bulla.

solution c (100 μ l) was administered to the left ear for both groups in the same way.

The animals were maintained in the prone position until waking up - to verify filling of the middle ear cavity.

3. Perfusion And Fixation:

14 days after the second surgery, animals were perfused transcardially with a PBS flush following by a fixative of 4% paraformaldehyde in 0.1M PBS. Immediately following the perfusion, the temporal bone was removed from the head. The bulla was opened and the cochlea was exposed. The apex was opened and the membrane of the round and oval windows was punched. The fixative solution was gently infused into the perilymphatic space through the apex hole and then allowed to flow out from windows. Then the cochleae were post-fixed in the same fixative solution for at least one day.

4. FITC-Phalloidin Staining of Hair Cells:

To identify and count hair cells in the organ of Corti, a direct immunostaining method was used to label the actin present naturally in the stereocilia bundles of the hair cells. The cochlea was dissected and the perilymphatic space was fully exposed. The samples were washed three times with PBS (1 ml per well) and permeabilized with 1% Triton X-100 in PBS for 10 minutes at room temperature. After three washes in PBS, The cochlea samples were incubated with FITC-labeled Phalloidin (1:60 from stock, i.e. 1.67 $\mu\text{g/ml}$ in concentration, 1 ml/well) for 45 minutes at room temperature. The plates were covered with aluminum foil as the Phalloidin is light sensitive. After three more washes with PBS, the labeled cochleas were then bisected and all four turns were removed by microdissection, preserving the hook portion of the basal turn. The turns were mounted on a coverslip (24x60 mm) with Vectashield mounting medium, covered with a glass coverslip and sealed with nail polish. The cochlea turns were observed under a Nikon Diaphot-300 inverted fluorescence microscope, using FITC filters

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and fluorescence optics. The missed outer hair cells (OHC) were counted in every 175 μ m segments (containing 20 OHCs in each row of OHC) beginning at the apex and continuing toward the base.

5. Determination of Hair Cell Number

The cochlea turns were observed under a Nikon Diaphot-300 inverted fluorescence microscope, using FITC filters and fluorescence optics. In each cochlea, the number of missed outer hair cells (OHC) was counted in each 175 μ m segments (containing 20 OHCs in each row of OHC) beginning from the apex and continuing toward the base. The numbers were filled in a cochleogram form for analysis the percentage of OHC loss in each rows, each turns and in whole cochlea of left and right ears. There are four turns per cochlea, the apex called turn 1 is counted top 3.5 mm in length, middle turns including turns 2 (counted 3.5mm-7.0 mm from apex) and turn 3 (7.0mm-10.5mm from apex), and the basal turn called turn 4 (10.5mm-14.0mm).

RESULTS

Table 1 and Figure 1-A show that there was a large and very significant difference in the number of OHCs loss between vehicle and GPI1046 treatments after exposure to ototoxins. In animals that received pretreatment of either 10 ng or 1 ng GPI1046, a significant ($p < 0.0001$, t-test) number of hair cells survived after exposure to ototoxins. Maximal protective activity was on the basal turns (Figure 1-B & C). The results indicate that under this experimental paradigm GPI1046 is capable to completely protect hair cells against ototoxicity.

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Table 1 GPI1046 Protects OHC Loss (%) in Intramiddle Ear Administered Neomycin-induced Hearing Loss Models

treatment time	Left - vehicle mean±SEM	Right- GPI 1046 () mean±SEM	t-test
10 ng GPI1046	86.78±6.81	11.44±7.27	p<0.0001
n=9	73.36± 1.12	15.47± 6.05	p<0.0001
turn-1	94.72± 5.59	13.93±10.75	p<0.0001
turn-2	90.10±10.50	11.64±10.85	p<0.0001
turn-3	88.94±11.73	4.69± 2.85	p<0.0001
turn-4			
1 ng GPI1046	72.14±11.19	3.86±0.37	p<0.0001
n=7	56.11± 9.90	8.85±1.47	p<0.0001
turn-1	72.96±13.97	4.01±0.53	p<0.0001
turn-2	74.07±11.59	0.92±0.10	p<0.0001
turn-3	85.43± 4.82	1.67±1.25	p<0.0001
turn-4			

The results indicate that intramiddle ear administered neomycin caused a marked disruption of the four-row-structure and the surviving cells were randomly located. While the treatment with neomycin and vehicle resulted in almost complete loss of hair cells in most animals, four of 19 animals only lost between ~10% to 37% of OHCs in the same manner (Figure 4-A, B).

Example 3

**Systemic Administered GPI-1046 Protect Hair Cells
Against Ototoxicity
Induced by Intramiddle Ear Administered Neomycin**

1. MATERIALS

The materials used in the following Example were same as described in the sample 1.

2. METHODS**A. Systemic Administration of GPI-1046:**

20 guinea pigs were treated either with GPI-1046 or vehicle prior to acceptance of ototoxin. Ten of them were subcutaneously injected with fresh made GPI-1046 solution. On the day of injection, 100 mg of GPI-1046 was dissolved in 1 ml of ethanol, then added 20% of the Intralipids solution into it to the final volume at 3 ml. The final GPI-1046 concentration was 10 mg/0.3 ml. Each animal was subcutaneously injected with 0.3 ml of GPI-1046 solution at day 0, day 2 and day 7 of the experimental schedule. Another 10 animals were subcutaneously injected with 0.3 ml of vehicle, 20% Intralipids, individually at day 0, day 2 and day 7 of the experimental schedule too.

B. Middle Ear Administration of Neomycin:

At the day 2, guinea pigs used in this study were administered neomycin or vehicle in middle ear.

Animals were anesthetized with intramuscular injection of a mixture of ketamine (80 mg/kg) and

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xylozine (4 mg/kg). Through a post-auricular incision, the right bulla was identified. A hole was drilled to open the middle ear cavity (care was taken not to injure the tympanic annulus or ossicles). A piece of gelfoam (~2mm³) was soaked with neomycin solution (fresh made at the concentration of 50 mg/ml) and was inserted into the round window niche. The remain neomycin solution (~100 µl) was then injected into the middle ear cavity. So total 5 mg of neomycin was applied to the right middle ear. The hole was covered with a piece of clear plastic sheet which was stuck on the skull with a superglue. The incision was closed with clippers. The same procedure was performed at the left ear, but administered with vehicle solution (100 µl of 0.9% saline) instead of neomycin. The animals were maintained in the prone until they woke up to verify filling of the cavity.

C. Perfusion And Fixation:

At the 16th day, animals were perfused transcardially with a PBS flush following by a fixative of 4% paraformaldehyde in 0.1M PBS. Immediately following the perfusion, the temporal bone was removed from the head. The bulla was opened and the cochlea was exposed. The apex was opened and the membrane of the round and oval windows was broken. The fixative solution was infused into the perilymphatic space of the cochlea, and the fixative solution was gently irrigated through the apex hole and then allowed to flow out from windows. Then the cochleae were post-fixed in the same fixative solution for at least one day.

D. FITC-Phalloidin Staining of Hair Cells:

Same in example 2

E. Determination of Hair Cell Number

Same as example 2

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3. RESULTS

A. Protection Effects of Systemic administered GPI-1046 against Neomycin-induced Hair Cell Loss:

Right ears (neomycin treated) from both groups - the vehicle treated and the GPI1046 treated animals were compared. There was a significant difference in the loss of hair cells between vehicle and GPI-1046 treatments (~31%, Figure 5). While neomycin in the vehicle treated animals induced about 75% of hair cell loss, GPI1046 treatment resulted in a loss that was around 45%. The significant protection effects of GPI-1046 was seen on the apex turns and top middle turns (Figure 6).

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CLAIMS

What is claimed is:

1. A method for treating hearing loss comprising administering to a subject a therapeutically effective amount of a neuroimmunophilin compound.
2. The method of claim 1, wherein the hearing loss is associated with injury or degeneration of neuroepithelial hair cells in the inner ear.
3. The method of claim 1, wherein the hearing loss is associated with injury or degeneration of spiral ganglion neurons.
4. The method of claim 1, wherein the neuroimmunophilin compound is a synthetic small molecule.
7. The method of claim 1, wherein the neuroimmunophilin compound is administered at a dose of about 1 ng/kg/day to about 20 mg/kg/day.
11. A method for treating lesions or disturbances to the vestibular apparatus comprising administering to a subject having such a lesion or disturbance a therapeutically effective amount of a neuroimmunophilin compound.
12. The method of claim 11, wherein the lesion or disturbance results in dizziness, vertigo or loss of balance.

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**METHOD FOR PREVENTING AND TREATING
HEARING LOSS USING NEUROIMMUNOPHILIN LIGANDS**

ABSTRACT

The present invention relates generally to methods for preventing and/or treating injury or degeneration of cochlear hair cells and spiral ganglion neurons by administering immunophilins ligands, and in particular the neuroimmunophilin family of small molecules. The invention relates more specifically to methods for treating sensorineural hearing loss as well as vestibular disorders and tinnitus.

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Protection effect of immunophilins against cisplatin toxicity

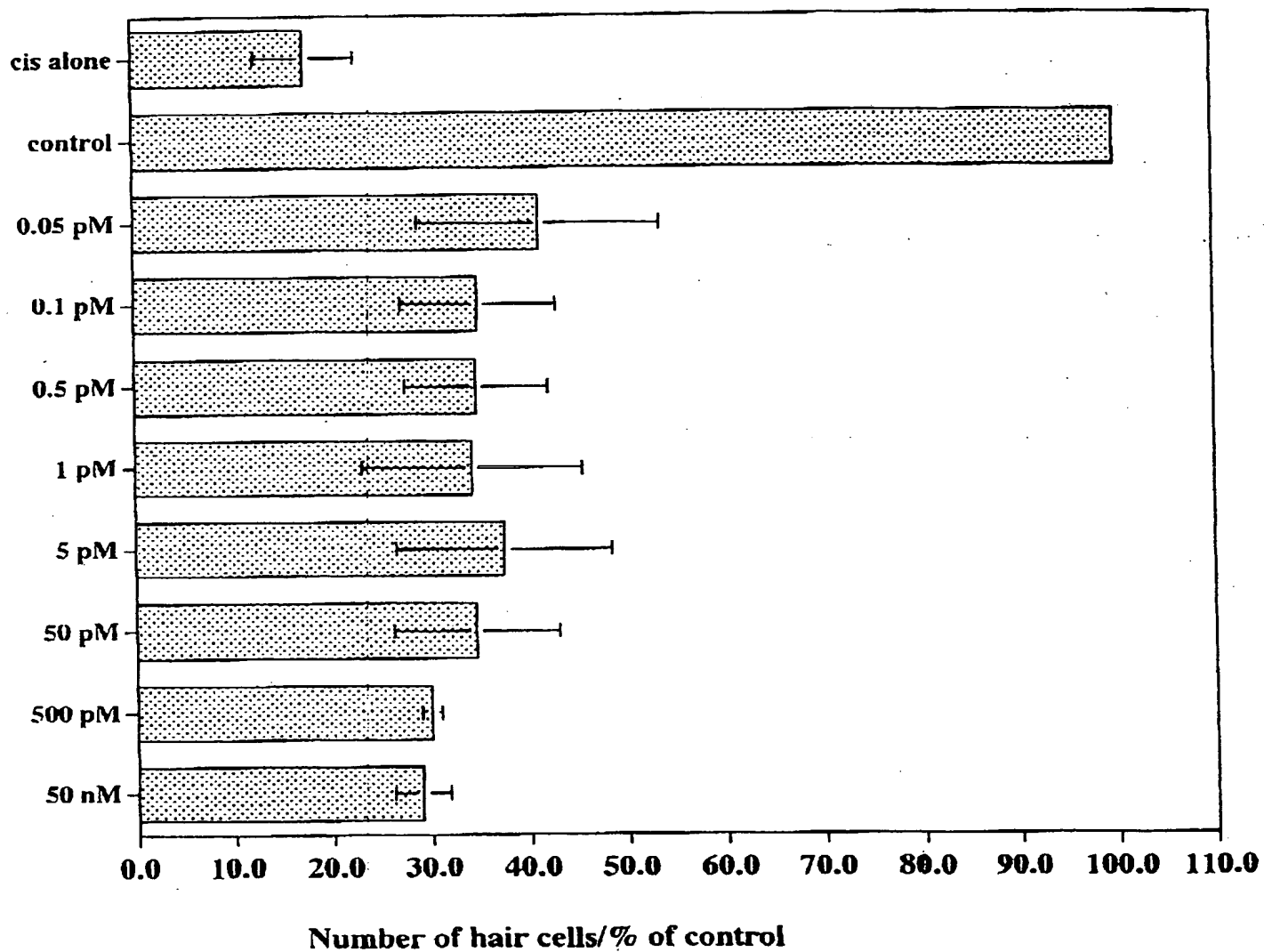


Figure ①

GPI1046 protects hair cells against neomycin toxicity

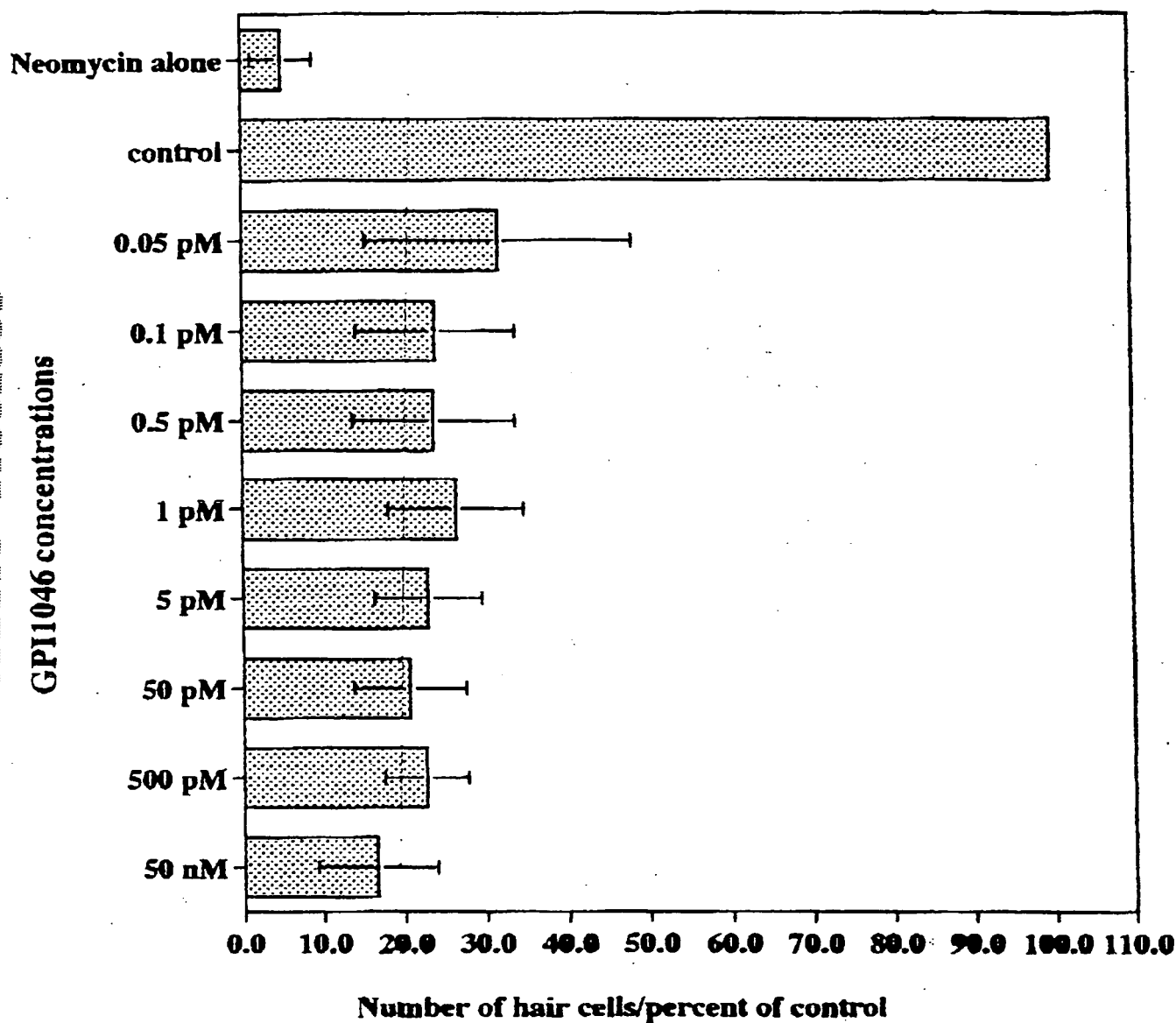
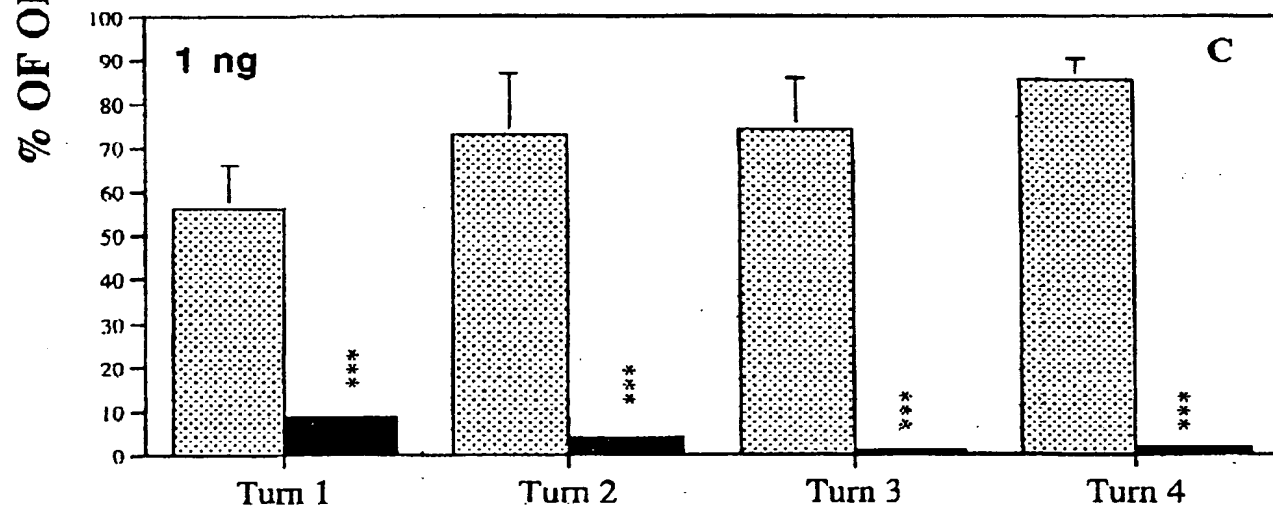
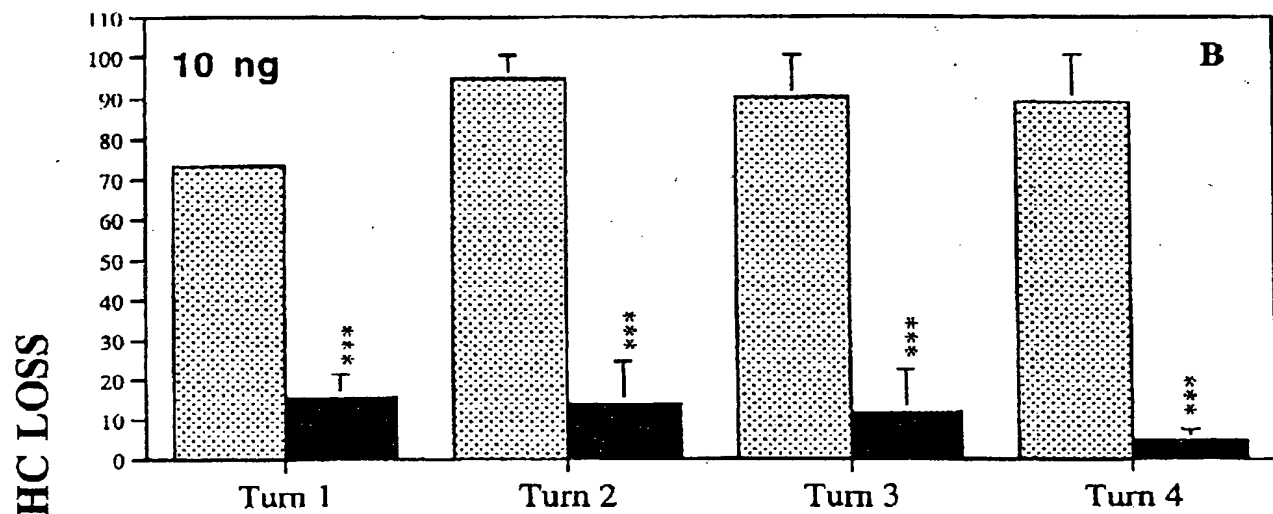
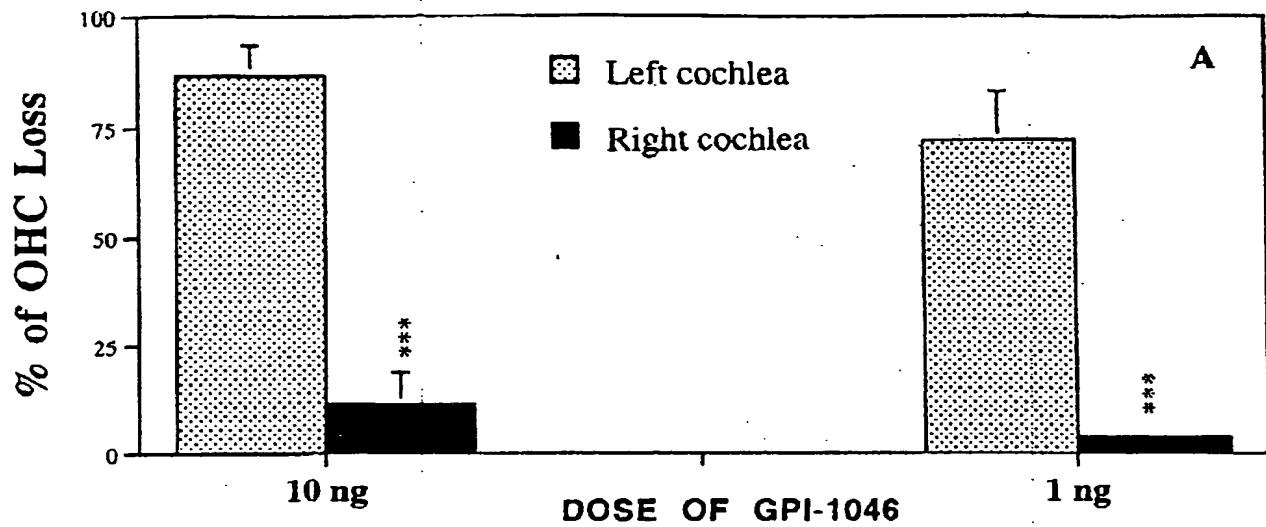
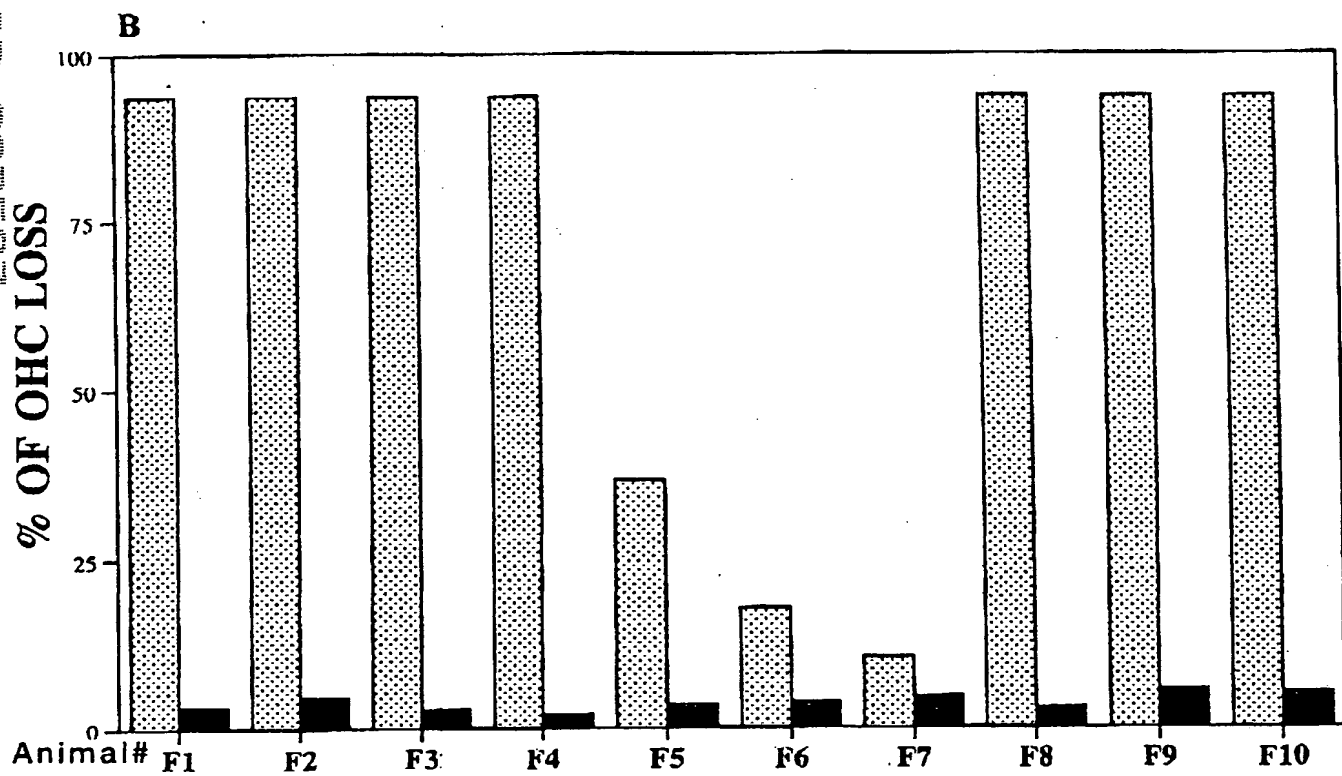
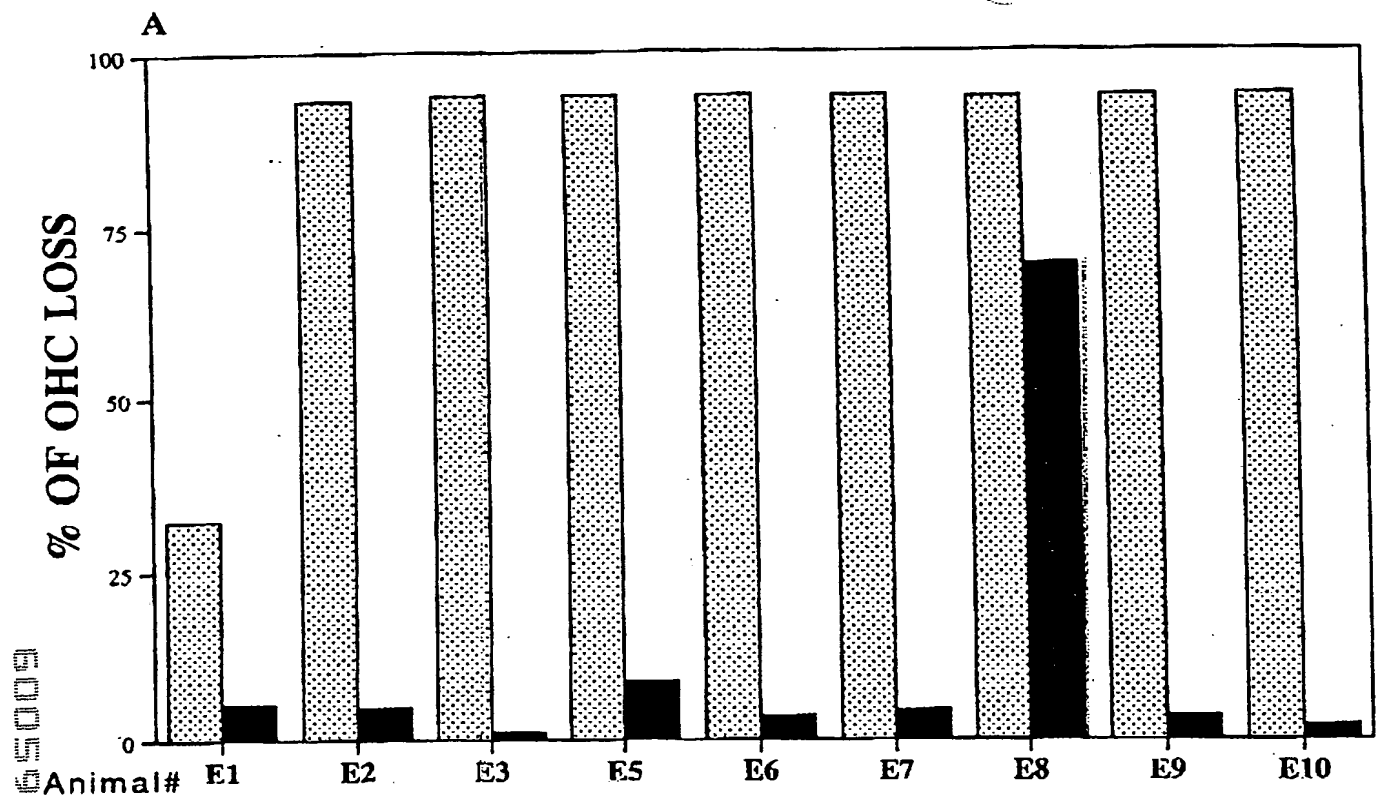


Figure 2



COCHLEAR TURNS

*** $p < 0.0001$, t-test



□ Left cochlea

■ Right cochlea

**Saline or Neomycin Applied to Round Window,
Vehicle or Fx Administered via S.C. Injection**

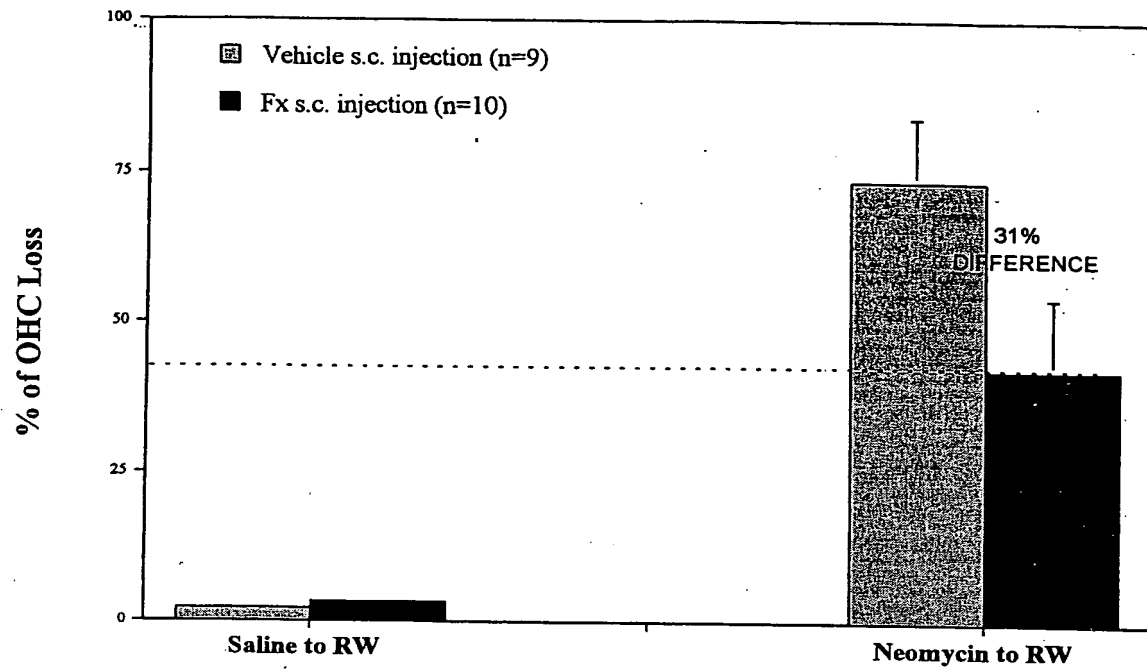


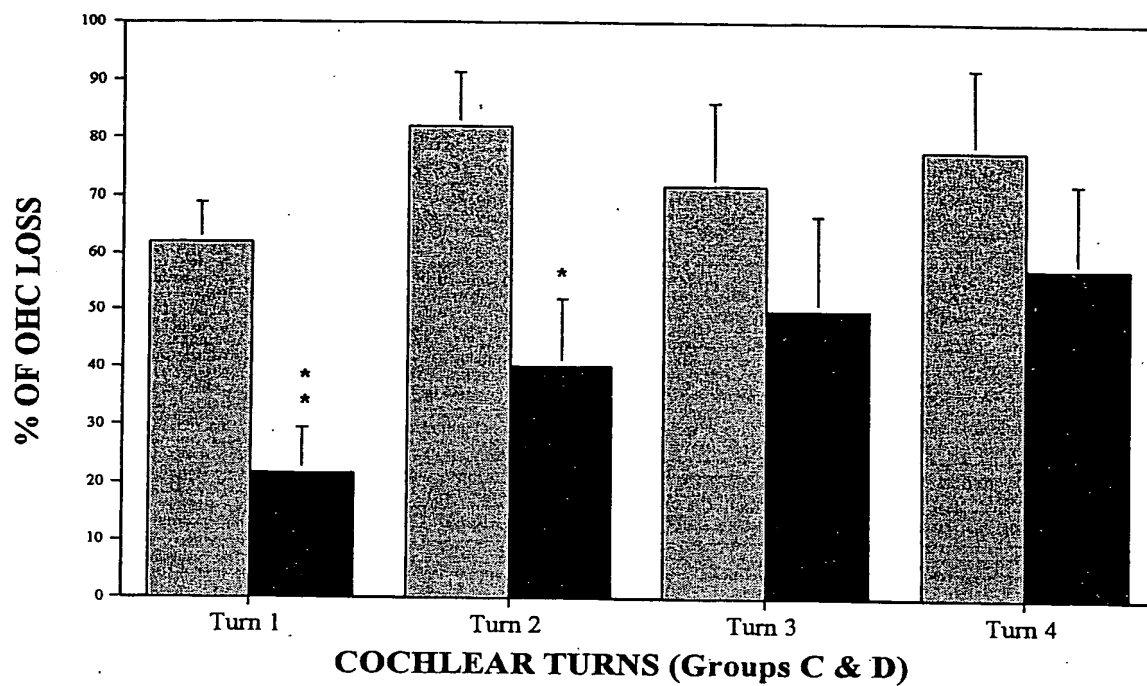
Figure 5

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**Neomycin Applied to Round Window, Vehicle
or Fx Administered via S.C. Injection**

▨ vehicle s.c. injection (n=9)

■ Fx s.c. injection (n=10)



** p<0.01

* p<0.05

t-test

Figure 6

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I. INSTANCES WHERE DOUBLE PATENT- ING ISSUE CAN BE RAISED

A double patenting issue may arise between two or more pending applications, between one or more pending applications and a patent, or between one or more pending applications and a published application. A double patenting issue may likewise arise in a reexamination proceeding between the patent claims being reexamined and the claims of one or more applications and/or patents. Double patenting does not relate to international applications which have not yet entered the national stage in the United States.

A. Between Issued Patent and One or More Applications

Double patenting may exist between an issued patent and an application filed by the same inventive entity, or by an inventive entity having a common inventor with the patent, and/or by the owner of the patent. Since the inventor/patent owner has already secured the issuance of a first patent, the examiner must determine whether the grant of a second patent would give rise to an unjustified extension of the rights granted in the first patent.

B. Between Copending Applications—Provisional Rejections

Occasionally, the examiner becomes aware of two copending applications filed by the same inventive entity, or by different inventive entities having a common inventor, and/or by a common assignee that would raise an issue of double patenting if one of the applications became a patent. Where this issue can be addressed without violating the confidential status of applications (35 U.S.C. 122), the courts have sanctioned the practice of making applicant aware of the potential double patenting problem if one of the applications became a patent by permitting the examiner to make a “provisional” rejection on the ground of double patenting. *In re Mott*, 539 F.2d 1291, 190 USPQ 536 (CCPA 1976); *In re Wetterau*, 356 F.2d 556, 148 USPQ 499 (CCPA 1966). The merits of such a provisional rejection can be addressed by both the applicant and the examiner without waiting for the first patent to issue.

The “provisional” double patenting rejection should continue to be made by the examiner in each application as long as there are conflicting claims in more than one application unless that “provisional” double patenting rejection is the only rejection remaining in one of the applications. If the “provisional” double patenting rejection in one application is the only rejection remaining in that application, the examiner should then withdraw that rejection and permit the application to issue as a patent, thereby converting the “provisional” double patenting rejection in the other application(s) into a double patenting rejection at the time the one application issues as a patent.

If the “provisional” double patenting rejections in both applications are the only rejections remaining in those applications, the examiner should then withdraw that rejection in one of the applications (e.g., the application with the earlier filing date) and permit the application to issue as a patent. The examiner should maintain the double patenting rejection in the other application as a “provisional” double patenting rejection which will be converted into a double patenting rejection when the one application issues as a patent.

C. Between One or More Applications and a Published Application - Provisional Rejections

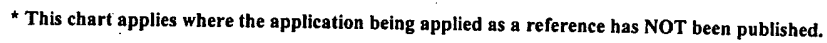
Double patenting may exist between a published patent application and an application filed by the same inventive entity, or by different inventive entities having a common inventor, and/or by a common assignee. Since the published application has not yet issued as a patent, the examiner is permitted to make a “provisional” rejection on the ground of double patenting. See the discussion regarding “provisional” double patenting rejection in subsection B. above.

common relationship of inventorship and/or ownership of two or more patents or applications. Since the doctrine of double patenting seeks to avoid unjustly extending patent rights at the expense of the public, the focus of any double patenting analysis necessarily is on the claims in the multiple patents or patent applications involved in the analysis.

There are generally two types of double patenting rejections. One is the "same invention" type double patenting rejection based on 35 U.S.C. 101 which states in the singular that an inventor "may obtain a patent". The second is the "nonstatutory-type" double patenting rejection based on a judicially created doctrine grounded in public policy and which is primarily intended to prevent prolongation of the patent term by prohibiting claims in a second patent not patentably distinguishing from claims in a first patent. Nonstatutory double patenting includes rejections based on one-way determination of obviousness and on two-way determination of obviousness. Nonstatutory double patenting could include a rejection which is not the usual "obviousness-type" double patenting rejection. This type of double patenting rejection is rare and is limited to the particular facts of the case. *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968).

Refer to Charts I-A, I-B, II-A, II-B, III-A, and III-B for an overview of the treatment of applications having conflicting claims (e.g., where a claim in an application is not patentably distinct from a claim in a patent or another application).

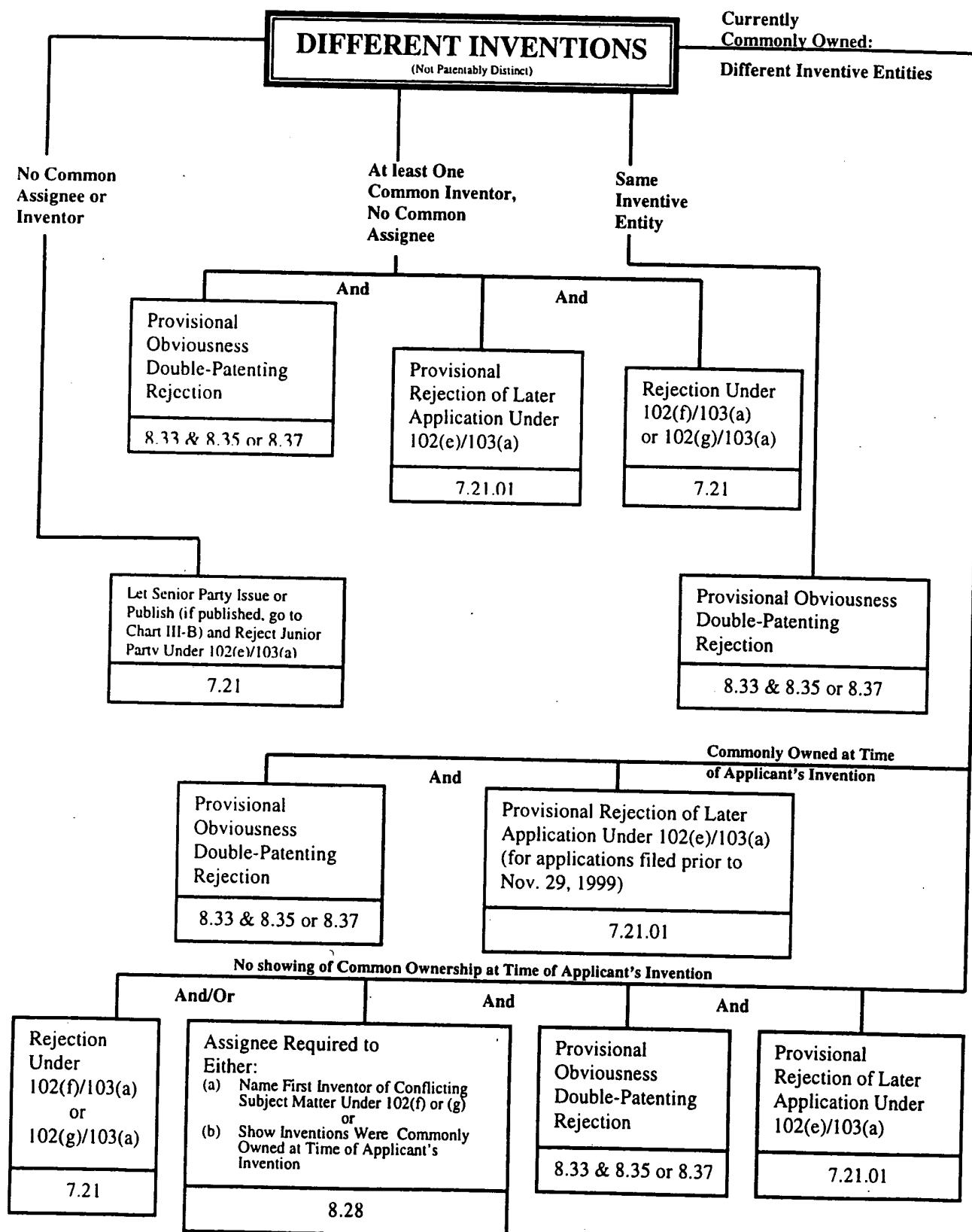
CHART I-A



* This chart applies where the application being applied as a reference has NOT been published.

CONFLICTING CLAIMS BETWEEN: TWO APPLICATIONS *

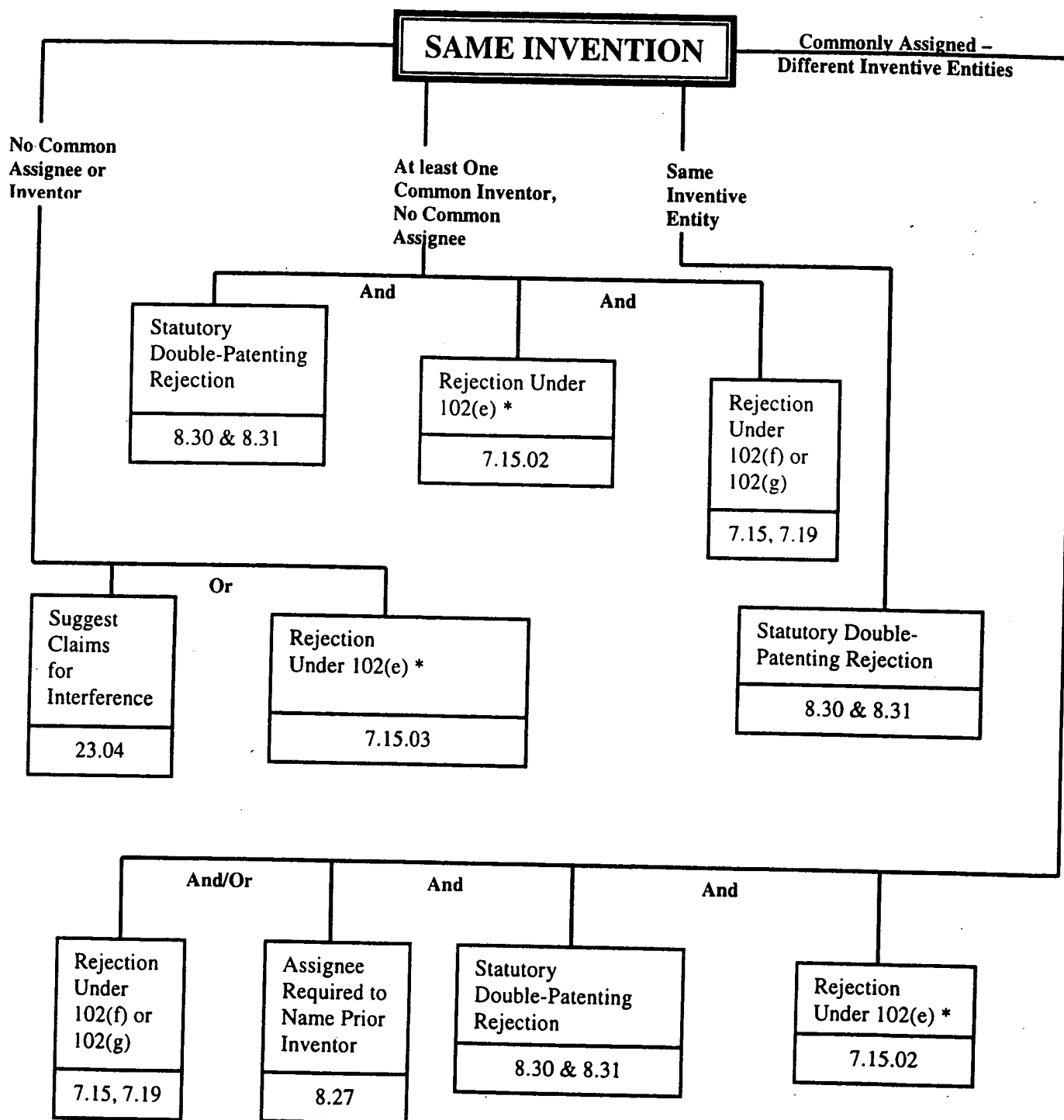
CHART I-B



* This chart applies where the application being applied as a reference has NOT been published.

CONFLICTING CLAIMS BETWEEN: APPLICATION AND A PATENT

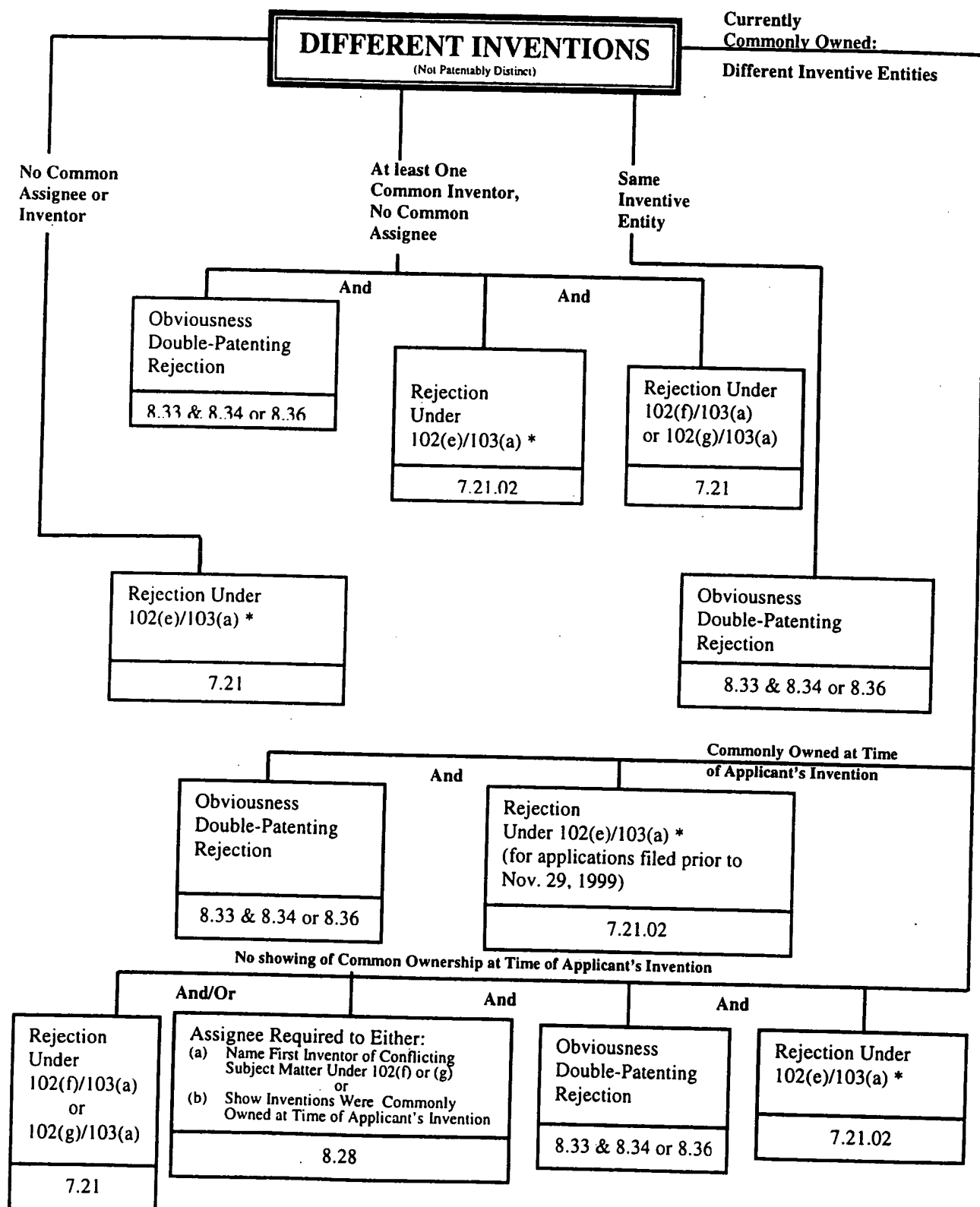
CHART II-A



* A rejection under 35 U.S.C. 102(e) would NOT be appropriate where the application being examined was filed on or after Nov. 29, 2000 or filed prior to Nov. 29, 2000 and voluntarily published, and the reference patent issued from an international application (reference patent has no 102(e) prior art effect).

CONFLICTING CLAIMS BETWEEN: APPLICATION AND A PATENT

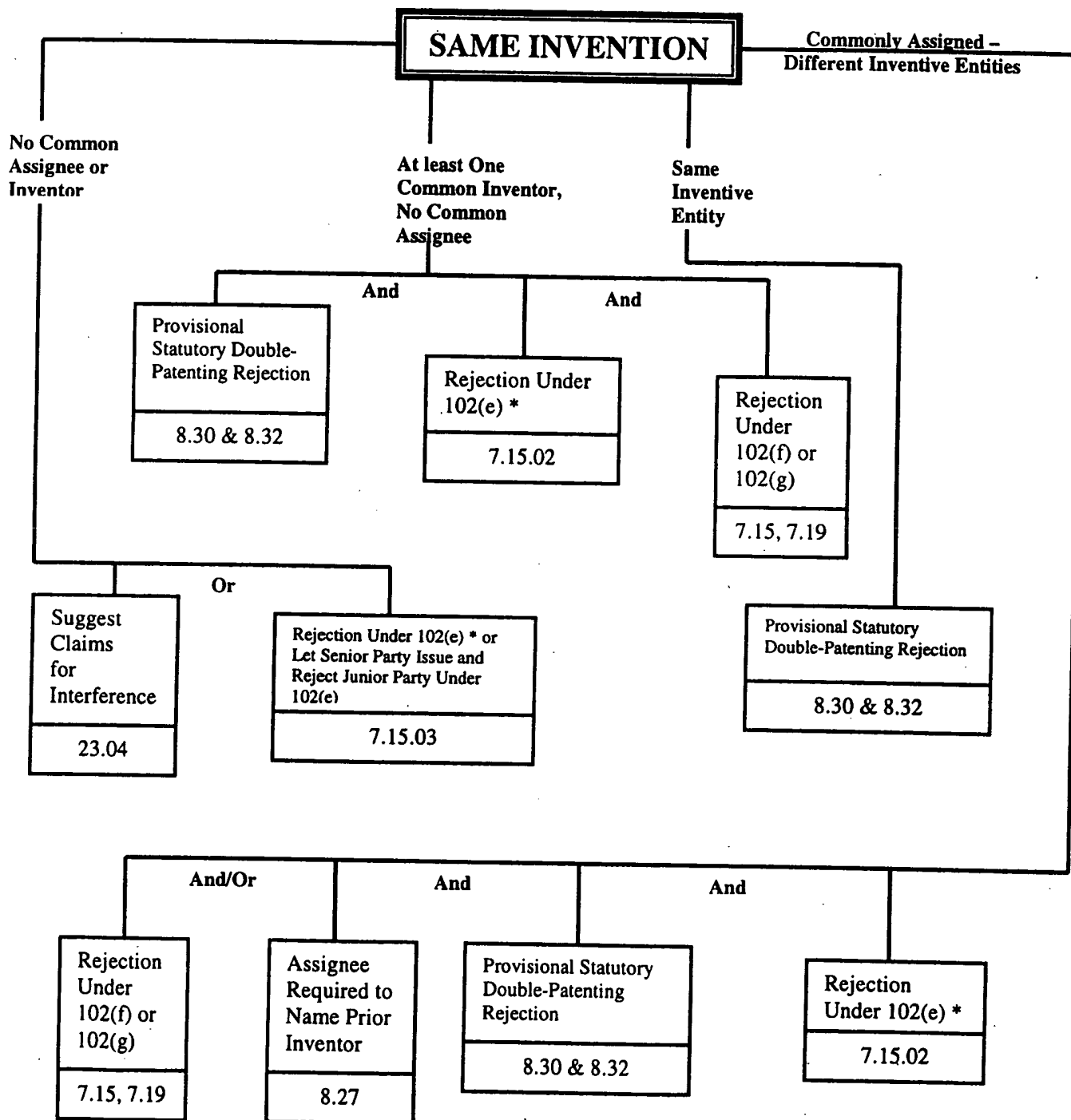
CHART II-B



*The reference patent would NOT be prior art under 35 U.S.C. 102(e) where the patent issued from an international application and the application being examined was filed on or after Nov. 29, 2000 or filed prior to Nov. 29, 2000 and voluntarily published.

**CONFLICTING CLAIMS BETWEEN:
APPLICATION AND A PUBLISHED
APPLICATION**

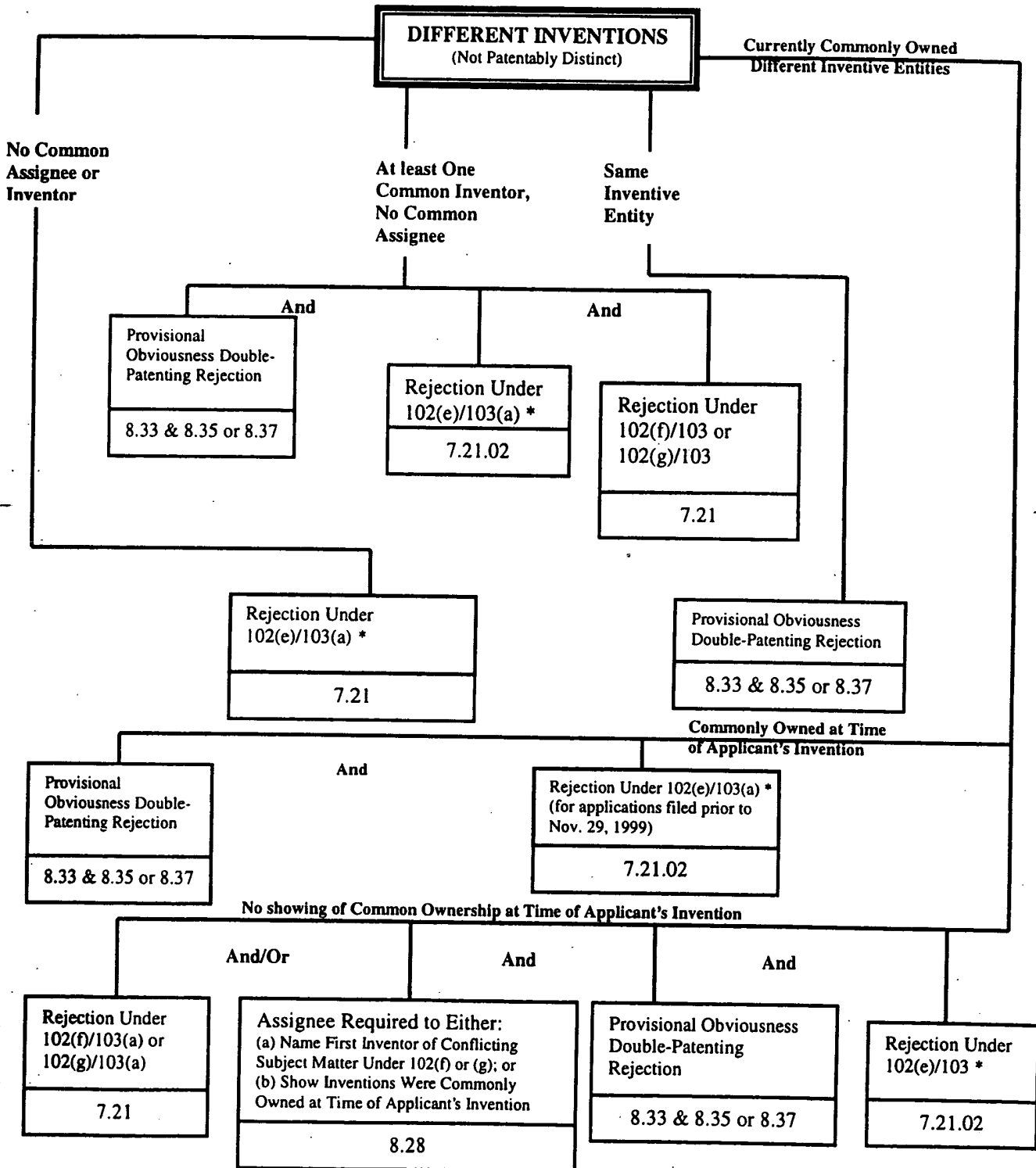
CHART III-A



* Apply 35 U.S.C. 102(e) rejection if the application being examined was filed on or after Nov. 29, 2000 or filed prior to Nov. 29, 2000 and voluntarily published, otherwise, apply rejection under 35 U.S.C. 102(a) or (b), where appropriate and make a "provisional" rejection of the later application under 35 U.S.C. 102(e) using form paragraph 7.15.01.

**CONFLICTING CLAIMS BETWEEN:
APPLICATION AND A PUBLISHED
APPLICATION**

CHART III-B



* Apply 35 U.S.C. 102(e)/103(a) rejection if the application being examined was filed on or after Nov. 29, 2000 or filed prior to Nov. 29, 2000 and voluntarily published, otherwise, apply rejection under 35 U.S.C. 102(a)/103(a) or 102(b)/103(a), where appropriate and make a "provisional" rejection of the later application under 35 U.S.C. 102(e)/103(a) using form paragraph 7.21.01.

(a) One-Way Obviousness

If the application at issue is the later filed application or both are filed on the same day, only a one-way determination of obviousness is needed in resolving the issue of double patenting, i.e., whether the invention defined in a claim in the application is an obvious variation of the invention defined in a claim in the patent. See, e.g., *In re Berg*, 46 USPQ2d 1226 (Fed. Cir. 1998) (the court applied a one-way test where both applications were filed the same day). If a claimed invention in the application is obvious over a claimed invention in the patent, there would be an unjustified timewise extension of the patent and an obvious-type double patenting rejection is proper. Unless a claimed invention in the application is obvious over a claimed invention in the patent, no double patenting rejection of the obvious-type should be made, but this does not necessarily preclude a rejection based on another type of nonstatutory double patenting (see MPEP § 804, paragraph II.B.2. below).

Similarly, even if the application at issue is the earlier filed application, only a one-way determination of obviousness is needed to support a double patenting rejection in the absence of a finding of: (A) administrative delay on the part of the Office causing delay in prosecution of the earlier filed application; and (B) applicant could not have filed the conflicting claims in a single (i.e., the earlier filed) application. See MPEP § 804, paragraph II.B.1.(b) below.

Form paragraph 8.33 and the appropriate one of form paragraphs 8.34 - 8.37 may be used to make nonstatutory rejections of the obvious-type.

(b) Two-Way Obviousness

If the patent is the later filed application, the question of whether the timewise extension of the right to exclude granted by a patent is justified or unjustified must be addressed. A two-way test is to be applied only when the applicant could not have filed the claims in a single application *and* there is administrative delay. *In re Berg*, 46 USPQ2d 1226 (Fed. Cir. 1998) ("The two-way exception can only apply when the applicant could not avoid separate filings, and even then, only if the PTO controlled the rates of prosecution to cause the later filed species claims to issue before the claims for a genus in an earlier application . . . In Berg's case, the two applications could have been filed as one, so it is irrelevant to our disposition who actually controlled the respective rates of prosecution."). In the absence of administrative delay, a one-way test is appropriate. *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993) (applicant's voluntary decision to obtain early issuance of claims directed to a species and to pursue prosecution of previously rejected genus claims in a continuation is a considered election to postpone by the applicant and not administrative delay). Unless the record clearly shows administrative delay by the Office and that applicant could not have avoided filing separate applications, the examiner may use the one-way obviousness determination and shift the burden to applicant to show why a two-way obviousness determination is required.

When making a two-way obviousness determination where appropriate, it is necessary to apply the *Graham* obviousness analysis twice, once with the application claims as the claims in issue, and once with the patent claims as the claims in issue. Where a two-way obviousness determination is required, an obvious-type double patenting rejection is appropriate only where each analysis compels a conclusion that the invention defined in the claims in issue is an obvious variation of the invention defined in a claim in the other application/patent. If either analysis does not compel a conclusion of obviousness, no double patenting rejection of the obvious-type is made, but this does not necessarily preclude a nonstatutory double patenting rejection based on the fundamental reason to prevent unjustified timewise extension of the right to exclude granted by a patent. *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968).

Although a delay in the processing of applications before the Office that would cause

patents to issue in an order different from the order in which the applications were filed is a factor to be considered in determining whether a one-way or two-way obviousness determination is necessary to support a double patenting rejection, it may be very difficult to assess whether an applicant or the administrative process is primarily responsible for a delay in the issuance of a patent. On the one hand, it is applicant who presents claims for examination and pays the issue fee. On the other hand, the resolution of legitimate differences of opinion that must be resolved in an appeal process or the time spent in an interference proceeding can significantly delay the issuance of a patent. Nevertheless, the reasons for the delay in issuing a patent have been considered in assessing the propriety of a double patenting rejection. Thus, in *Pierce v. Allen B. DuMont Laboratories, Inc.*, 297 F.2d 323, 131 USPQ 340 (3d. Cir. 1961), the court found that administrative delay may justify the extension of patent rights beyond 17 years but “a considered election to postpone acquisition of the broader [patent after the issuance of the later filed application] should not be tolerated.” In *Pierce*, the patentee elected to participate in an interference proceeding [after all claims in the application had been determined to be patentable] whereby the issuance of the broader patent was delayed by more than 7 years after the issuance of the narrower patent. The court determined that the second issued patent was invalid on the ground of double patenting. Similarly, in *In re Emert*, 124 F.3d 1458, 44 USPQ2d 1149 (Fed. Cir. 1997), the court found that the one-way test is appropriate where applicants, rather than the Office, had significant control over the rate of prosecution of the application at issue. In support of its finding that the applicants were responsible for delaying prosecution of the application during the critical period, the court noted that the applicants had requested and received numerous time extensions in various filings. More importantly, the court noted, after initially receiving an obviousness rejection of all claims, applicants had waited the maximum period to reply (6 months), then abandoned the application in favor of a substantially identical continuation application, then received another obviousness rejection of all claims, again waited the maximum period to reply, and then again abandoned the application in favor of a second continuation application substantially identical to the original filing. On the other hand, in *General Foods Corp. v. Studiengesellschaft Kohle mbH*, 972 F.2d 1272, 23 USPQ2d 1839 (Fed. Cir. 1992), the court elected not to hold the patentee accountable for a delay in issuing the first filed application until after the second filed application issued as a patent, even where the patentee had intentionally refiled the first filed application as a continuation-in-part after receiving a Notice of Allowance indicating that all claims presented were patentable. Similarly, where, through no fault of the applicant, the claims in a later filed application issue first, an obvious-type double patenting rejection is improper, in the absence of a two-way obviousness determination, because the applicant does not have complete control over the rate of progress of a patent application through the Office. *In re Braat*, 937 F.2d 589, 19 USPQ2d 1289 (Fed. Cir. 1991). While acknowledging that allowance of the claims in the earlier filed application would result in the timewise extension of an invention claimed in the patent, the court was of the view that the extension was justified under the circumstances in this case, indicating that a double patenting rejection would be proper only if the claimed inventions were obvious over each other — a two-way obviousness determination.

Form paragraph 8.33 and the appropriate one of form paragraphs 8.34-8.37 may be used to make nonstatutory rejections of the obvious type.

¶ 8.33 Basis for Nonstatutory Double Patenting, “Heading” Only

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed.

applications filed on or after November 29, 1999, rejections under 35 U.S.C. 102(e)/103(a) should not be made or maintained if the applicant provides evidence that the subject matter of the reference and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

2. Another Type of Nonstatutory Double Patenting Rejection

There are some unique circumstances where it has been recognized that another type of nonstatutory double patenting rejection is applicable even where the inventions claimed in two or more applications/patents are considered nonobvious over each other. These circumstances are illustrated by the facts before the court in *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). In affirming the double patenting rejection, the court summed up the situation:

in appellant's own terms: The combination ABC was old. He made two improvements on it, (1) adding X and (2) adding Y, the result still being a unitary clip of enhanced utility. While his invention can be practiced in the forms ABCX or ABCY, the greatest advantage and best mode of practicing the invention as disclosed is obtained by using both inventions in the combination ABCXY. His first application disclosed ABCXY and other matters. He obtained a patent claiming [a clip comprising] BCX and ABCX, . . . so claiming these combinations as to cover them *no matter what other feature is incorporated in them*, thus covering effectively ABCXY. He now, many years later, seeks more claims directed to ABCY and ABCXY. Thus, protection he already had would be extended, albeit in somewhat different form, for several years beyond the expiration of his patent, were we to reverse.

397 F.2d at 355-56, 158 USPQ at 216 (emphasis in original).

The court recognized that "there is no double patenting in the sense of claiming the same invention because ABCX and ABCY are, in the technical patent law sense, different inventions. The rule against double patenting,' however, is not so circumscribed. The fundamental reason for the rule is to *prevent unjustified timewise extension of the right to exclude* granted by a patent no matter how the extension is brought about. To . . . prevail here, appellant has the burden of establishing that the invention claimed in his patent is independent and distinct' from the invention of the appealed claims. . . . appellant has clearly not established the independent and distinct character of the inventions of the appealed claims." 397 F.2d at 354-55, 158 USPQ at 214-15 (emphasis in original). The court observed:

The controlling fact is that patent protection for the clips, fully disclosed in and covered by the claims of the patent, would be extended by allowance of the appealed claims. Under the circumstance of the instant case, wherein we find no valid excuse or mitigating circumstances making it either reasonable or equitable to make an exception, and wherein there is no terminal disclaimer, the rule against "double patenting" must be applied.

397 F.2d at 355, 158 USPQ at 215.

The decision in *In re Schneller* did not establish a rule of general application and thus is limited to the particular set of facts set forth in that decision. The court in *Schneller* cautioned "against the tendency to freeze into rules of general application what, at best, are statements applicable to particular fact situations." *Schneller*, 397 F.2d at 355, 158 USPQ at 215. Nonstatutory double patenting rejections based on *Schneller* **will be rare**. The Technology Center (TC) Director must approve any nonstatutory double patenting rejections based on *Schneller*. If an examiner determines that a double patenting rejection based on *Schneller* is appropriate in his or her application, the examiner should

first consult with his or her supervisory patent examiner (SPE). If the SPE agrees with the examiner then approval of the TC Director must be obtained before such a nonstatutory double patenting rejection can be made.

A fact situation similar to that in *Schneller* was presented to a Federal Circuit panel in *In re Kaplan*, 789 F.2d 1574, 229 USPQ 678 (Fed. Cir. 1986). Kaplan had been issued a patent on a process of making chemicals in the presence of an organic solvent. Among the organic solvents disclosed and claimed as being useful were tetraglyme and sulfolane. One unclaimed example in the patent was specifically directed to a mixture of these two solvents. The claims in the application to Kaplan and Walker, the application before the Office, were directed to essentially the same chemical process, but requiring the use of the solvent mixture of tetraglyme and sulfolane. In reversing the double patenting rejection, the court stated that the mere fact that the broad process claim of the patent requiring an organic solvent reads on or "dominates" the narrower claim directed to basically the same process using a specific solvent mixture does not, *per se*, justify a double patenting rejection. The court also pointed out that the double patenting rejection improperly used the disclosure of the joint invention (solvent mixture) in the Kaplan patent specification as though it were prior art.

A significant factor in the *Kaplan* case was that the broad invention was invented by Kaplan, and the narrow invention (i.e., using a specific combination of solvents) was invented by Kaplan and Walker. Since these applications (as the applications in *Braat*) were filed before the Patent Law Amendments Act of 1984 (Pub. Law 98-622, November 8, 1984) amending 35 U.S.C. 116 to expressly authorize filing a patent application in the names of joint inventors who did not necessarily make a contribution to the invention defined in each claim in the patent, it was necessary to file multiple applications to claim both the broad and narrow inventions. Accordingly, there was a valid reason, driven by statute, why the claims to the specific solvent mixture were not presented for examination in the Kaplan patent application.

Each double patenting situation must be decided on its own facts.

Form paragraph 8.33 and the appropriate one of form paragraphs 8.38 (between an issued patent and one or more applications) and 8.39 (provisional rejections) may be used to make this type of nonstatutory double patenting rejection.

¶ 8.38 Double Patenting - Non-Statutory (Based Solely on Improper Timewise Extension of Patent Rights) With a Patent

Claim [1] rejected under the judicially created doctrine of double patenting over claim [2] of U. S. Patent No. [3] since the claims, if allowed, would improperly extend the "right to exclude" already granted in the patent.

The subject matter claimed in the instant application is fully disclosed in the patent and is covered by the patent since the patent and the application are claiming common subject matter, as follows: [4]

Furthermore, there is no apparent reason why applicant was prevented from presenting claims corresponding to those of the instant application during prosecution of the application which matured into a patent. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

Examiner Note

1.

This form paragraph should only be used where approval from the TC Director to make a nonstatutory double patenting rejection based on *In re Schneller* has been obtained.

2.